

Study of the Adipocytokine visfatin in Obesity and Type 2 Diabetes Mellitus

Thesis

Submitted for the partial Fulfillment of MD degree in Endocrinology and
Metabolism

By

Eman Mohamed Fahmy

MSc. Endocrinology & Metabolism
Ain Shams University

Supervised of Under
prof.

Dr. Salah El Din Ahmed Shelbaya

Professor of Internal Medicine and Endocrinology
Faculty of Medicine
Ain Shams University

Prof

Dr./Nehad Shokry shoeib

Professor of Internal Medicine and Endocrinology
Faculty of Medicine
Ain Shams University

Ass.Prof. Salwa Seddik Hosney

Professor of Internal Medicine and Endocrinology
Lectuer of Internal Medicin and Endocrinology
Faculty of Medicine
Ain Shams University

Dr.Khalid Mahmoud Mackboul

Lectuer of Internal Medicin and Endocrinology
Faculty of Medicine
Ain Shams University

Dr Rania Sayed Abd el Baki

Lectuer of Internal Medicin and Endocrinology
Faculty of Medicine
Ain Shams University

2010

Acknowledgment

*I am greatly honored to express my deep thanks and gratitude to **Prof.Dr. Salah El Din Ahmed Shelbaya** Professor of internal medicine and endocrinology, Faculty of medicine, Ain Shams University, for his continuous support and guidance, valuable suggestions, expert advice and generous help which have greatly helped me to complete this work.*

*Also my profound gratitude to **Prof.Dr. Nehad Shokry Shoeib** Professor of internal medicine, Faculty of medicine, Ain Shams University, for her kind supervision, great support it was great honor to work under her supervision.*

*I would like to express my profound gratitude to **Dr. Salwa Seddik Hosney** Assistant Professor of internal medicine, Faculty of medicine, Ain Shams University, for her kind supervision, great support it was great honor to work under her supervision.*

*Also my profound gratitude to **Dr. Khalid Mahmoud Mackboul** Lecturer of internal medicine, Faculty of medicine, Ain Shams University, for their kind supervision, great support it was great honor to work under her supervision.*

*Also my profound gratitude to **Dr. Rania Sayed Abd El Baki** Lecturer of internal medicine, Faculty of medicine, Ain*

Shams University, for their kind supervision, great support it was great honor to work under her supervision.

*I would like to state great appreciation to **Prof.Dr.Hisham Mahmoud** Professor of diagnostic radiology, Faculty of medicine, Ain Shams University, for her kind support and help during this work,*

*Also I would like to state great appreciation to **Prof.Dr. Iman El-Ghohary** Professor of clinical pathology, Faculty of medicine, Ain Shams University, for her kind support and help during this work,*

Lastly, I want to thank all my family members and my patients without their help, this work could not have been completed.

List of Contents

	page
List of abbreviation	4
List of figures	9
List of tables	10
introduction	11
Aim of the work	13
Review of literature	
Chapter 1: Obesity	14
Chapter 2: Metabolic syndrome	39
Chapter 3: Type 2 diabetes mellitus	53
Chapter 4: Adipokines, Obesity and Insulin Resistance	70
Chapter 5: Visfatin	81
Subjects and methods	104
Results	116
Discussion	141
Summary and conclusion	153
Recommendation	156
References	157
Arabic summary	

List of Abbreviation

ACC	Acetyl-CoA carboxylase
ADIPO R2	Adiponectin receptor 2
AGTR	Agouti Related peptide
AIDS	Acquired immune deficiency syndrome
AKT	Members of Protein kinase B
ALI	Acute lung Injury
AMPK	5' adenosine monophosphate-activated protein kinase
APOA-IV	Apo-lipoprotein A- IV
AR	Adrenergic Receptors
ATP	Adenosine Tri Phosphate
ATP III	Adult treatment panel III
BAT	Brown Adipose Tissue
BDNF	Brain-Derived Neurotrophic Factor
BMI	Body Mass Index
BMR	Basal Metabolic Rate
BNP	Brain Naturetic Peptide
BP	Blood Pressure
CCL2	Chemokine (C-C motif) ligand 2
CGRP	Calcitonin Gene Related peptide
CHD	Coronary Heart Disease
CNS	Central Nervous System
CPE	Carboxy Peptidase E
CRH	Cortico trophin Releasing Hormone
CRP	C-Reactive Protein
CT	Computerized tomography
CVS	Cardiovascular disease
DBP	Diastolic Blood Pressure
DBP	Diastolic blood pressure
DM	Diabetes mellitus
DoHaD	Developmental origins of health and disease
EDHF	Endothelium Derived Hyperpolarizing Factor

EGIR	European Group for the study of Insulin Resistance
ELISA	Enzyme Linked Immune Assay
ENPP1	Ectonucleotide pyrophosphatase/phosphodiesterase 1
ERKs	Extracellular Signal-Regulated kinases
ETC	Electron Transport Chain
FFA	Free Fatty Acid
FIZZ3	Found in inflammatory zone 3
G6Pase	Glucose 6 phosphatase
G6PDH	Glucose 6 phosphate dehydrogenase
GDM	Gestational diabetes
GH	Growth Hormone
GLP-1	Glucagon like peptide 1
GLUT	Glucose transporter
HAART	Highly Active Antiviral Therapy
HDL	High Density Lipoprotein
HIV	Human immunodeficiency virus
HNF4A	Hepatocyte nuclear factor 4 alpha
HOMA	Homeostatic Model Assessment of insulin
HOMA(IR)	Homeostatic Model Assessment of Insulin Resistance
HPA	Hypothalamo Pituitary Axis
HSC	Hepatic Stellate Cells
HSL	Hormone Sensitive Lipase
HTN	Hypertension
IAPP	Islet Amyloid Poly Peptide
IBW	Ideal Body weight
IDF	International Diabetes Federation.
IFG	Impaired Fasting Glucose
IFG	Impaired fasting glucose
IGT	Impaired Glucose Tolerance
IHD	Ischemic Heart Disease
IKK	Inflammatory Kinase inhibitor $\kappa\beta$ Kinase
IKβ	Inhibitor of $\kappa\beta$
IL	Interleukin
IR	Insulin Resistance
IRS-1	Insulin Receptor Substrate-1

IUGR	Intra Uterine Growth Retardation
JNK	c-Jun NH3 terminal kinase
KCNJ11	ATP-sensitive potassium channel
KIR 6.2	Potassium Inward rectifier 6.2
KO	Knock Out
LC-CoA	long-chain coenzyme A
LDL	Low Density Lipoprotein
LDLR	Low Density Lipoprotein Receptor
LPL	Lipoprotein lipase
LVH	Left Ventricular Hypertrophy
LVM	Left Ventricular Mass
MAPK	Mitogen activated Protein Kinase
MC	MelanoCortin
MCF-7	Michigan Cancer Foundation - 7
MCP-1	Monocyte chemotactic protein-1
MCR	Melanocortin Receptor
MODY	Maturity onset diabetes of the young
MRS	Magnetic Resonance Spectroscopy
MSH	Melanocyte Stimulating Hormone
MSX	Metabolic Syndrome
NAD	Nicotin amide dinucleotide
NAFLD	Non Alcoholic Fatty Liver Disease
NAM	Nicotine amide mononucleotide
NamPRTase	Nicotine amide phoshop ribosyl transferase
NASH	Non Alcoholic Steatohepatitis
NCEP	National Cholesterol Education program
NEFA	Non Esterified free fatty acid
NF-κB	Nuclear Factor- Kappa Beta
NGF	Nerve Growth Factor
NGT	Normal Glucose Tolerance
NIY5	Neuron peptide Y 5
Ob	Obese gene
OGTT	Oral Glucose Tolerance Test
OLETF	Otsuka Long Evans Tokushima Fatty
OSA	Obstructive Sleep Apnea

PAI-I	Plasminogen Activator Inhibitor 1
PASP	Pulmonay Artery Systolic Pressure
PBEF	Pre Beta colony Enhancing Factor
PCOS	Polycystic Ovarian Syndrome
PDX-1	Pancreas Duodenum Homeobox-1
PET	Positron Emission Tomography
PGC1	Peroxisome proliferator-activated receptor Gamma coactivator 1
PI 3-kinases	Phosphoinositide 3-kinases
PI-3K	Phosphatidyl Inositol 3 Kinase
PKCE	Protein Kinase Carboxy Peptidase E
POMC	ProOpioMelanoCortin
PPARγ	Peroxisome Proliferators Activator Receptor γ
PTP1B	protein tyrosine-phosphatase 1B
RBP -4	Retinol Binding Protein -4
RIA	Radio Immuno Assay
RMR	Resting Membrane Rate
RNS	Reactive Nitrogen Species
ROS	Reactive oxygen Species
RXR	Retinoid X Receptor.
SBP	Systolic blood pressure
SHBG	Sex Hormone Binding Globulin
Sir2	sirtuin genes
SNPs	Single nucleotide polymorphism
SOCS	Suppressor of cytokines signaling
SPARC	Secreted Protein Rich in Cysteine
STAT	Signal Transducer and activator of transcription
SUR 1	Sulfonylurea Receptor1
TAG	Tri Acyl Glycerol
TCF7L2	Transcription factor 7-like 2
TG	Triglycerides
THP1	Human acute monocytic leukemia cell line
TNFα	Tumor necrosis Factor α
TrkA	Tyrosine Kinase A
Trp 64 Arg	Tryptophan in position 64 to Arginine

TZDs	Thiazolidinediones
UCP-1	Uncoupling Protein -1
UCP-2	Uncoupling Protein -2
UTI	Urinary tract infection
VLDL	Very low Density Lipoprotein
vWF Ag	Von –Willebrand factor antigen
WAT	White adipose tissue
WC	Waist Circumference.
WHO	World Health Organization
WHR	Waist Hip Ratio
WT	Wild Type
α AR	Alpha 1 adrenergic receptor
β 2 AR	Beta 2 adrenergic receptor
β 3 AR	Beta 3 adrenergic receptor

List of figures

Figure 1: A tentative model showing how changes in the structure of genes controlling important actions in human adipose tissue	19
Figure 2: hyperbolic relationship between beta cell function and insulin sensitivity in subjects with normal glucose tolerance	39
Figure 3: schematic representation of normal effects of insulin secretion	40
Figure 4: insulin signalling and insulin resistance.	41
Figure 5: insulin secretion schematic representation of normal glucose-induced insulin secretion;.....	48
Figure 6: Schematic representation of possible negative influence of hyperglycemia and increased NEFA, and of various modulators involved in insulin resistance	50
Figure 7: Schematic diagram of the metabolic syndrome metabolic syndrome with suggested mechanisms linking the metabolic syndrome components.....	68
Figure 8: Schematic diagram showing factors that stimulate or inhibit visfatin expression in visceral adipose tissue.....	93
Figure 9: Mean values of visfatin in the subgroups.	134
Figure 10: Mean values BMI in the studied subgroups.....	134
Figure 11: Mean values of fasting plasma glucose in the studied subgroups.	135
Figure 12: Mean values HbA1 C in the studied subgroups.....	135
Figure 13: Mean values HDL-C, Triglycerides .LDL-C, Cholesterol in the studied subgroups.	136
Figure 14: Mean values of Insulin, HOMA-IR in the studied subgroups. ...	136
Figure 15: The difference between subcutaneous and visceral fat in diabetic lean patient	137
Figure 16: The difference between subcutaneous and visceral fat in diabetic obese patient.....	138
Figure 17: Correlation between visfatin and BMI in the studied group.....	139
Figure 18: Correlation between visfatin and Waist circumference in the studied groups.	139
Figure 19: Correlation between visfatin and FPS in the studied group.....	140
Figure 20: Correlation between visfatin and triglyceride in the studied groups.	140
Figure 21: Correlation between visfatin and HbA1C in the studied groups.....	141
Figure 22: Correlation between visfatin and HOMA-IR in all the studied groups.	141

List of Tables

Table 1: The International Classification of adult underweight overweight and obesity according to BMI Classification of adults according to BMI (WHO, 1995, 2004).....	15
Table 2: Proposed Criteria for Clinical Diagnosis of Metabolic Syndrome...	58
Table 3: Comparison between the diabetic group (I) and control group (II) as regard all measured parameters.....	122
Table 4: Comparison between the obese group and lean group as regard all measured parameters.	123
Table 5:- Comparison between the studied groups (no.20) as regard all measured parameters.	124
Table 6: Comparison between the studied groups as regard VAT and SAT	125
Table 7: Comparison between the control group and diabetic group as regard VAT &SAT.....	125
Table 8: Comparison between the obese group and control group as regard VAT &SAT.....	125
Table 9: Comparison between the diabetic obese group and diabetic lean group as regard all measured parameters.	126
Table 10: Comparison between the diabetic obese group (I A) and control obese group (II A) as regard all measured parameters.....	127
Table 11: Comparison between the diabetic lean group and control lean group as regard all measured parameters	128
Table 12: Comparison between the control obese group and control lean group as regard all measured parameters	129
Table 13: Comparison between the diabetic obese group and diabetic lean group as regard VAT and SAT.	130
Table 14: Comparison between the diabetic obese group and control obese group as regard VAT and SAT	130
Table 15: Comparison between the diabetic lean group and control lean group as regard VAT and SAT.....	131
Table 16: Comparison between the control obese group and control lean group as regard VAT and SAT	131
Table 17: Person Correlation between the visfatin and all measured parameters.	132
Table 18: linear regression between the visfatin and all measured parameters	133

Introduction

Excess Adiposity is the most important risk in the development of insulin resistance and type 2 diabetes mellitus (**Bloomgarden, 2002**). Adipose tissue produces several proteins (adipocytokines) such as leptin, adiponectin, resistin, TNF α , and IL-6, that modulate insulin sensitivity and appear to play an important role in the pathogenesis of insulin resistance, diabetes, dyslipidemia, inflammation, and atherosclerosis (**Kershaw et al., 2004**).

Several adipokines including adiponectin, leptin, and interleukin (IL)-6, have been linked to the development of diabetes (**Thorand et al., 2005**).

In most studies, low adiponectin (**Lindsay et al., 2002; Kanaya et al., 2006**) and elevated IL-6 have been associated with the development of subsequent diabetes independent of obesity, and some have shown the associations to be independent of measures of insulin (**Duncan et al., 2004**).

However, the mechanisms by which fat tissue induces insulin resistance and the role of adipocytokines in the pathogenesis of Type 2 Diabetes Mellitus has not been well established (**Chen et al., 2006**).

In 2004, Fukuhara et al., identified a molecule that is expressed at much higher levels in visceral than in subcutaneous fat which was named Visfatin (**Fukuhara et al., 2005**).

Visfatin, also known as pre-B cell colony-enhancing factor, is a cytokine that is highly expressed in visceral fat and was originally isolated as a secreted factor that synergizes with IL-6 and stem cell (**Kralisch et al., 2005b**).

Visfatin was reported to be expressed almost exclusively in visceral adipose tissue and has insulin-like metabolic effects (**Fukuhara et al., 2005**).

It turns out that the molecule was previously identified as a growth factor for early B-lymphocytes termed pre-B cell colony enhancing factor (PBEF) (**Samal et al., 1994**).

The visfatin gene is expressed in adipocytes, where it is subject to regulation (**Kralisch et al., 2005b**).

Furthermore, in humans the gene is expressed predominantly in visceral adipose tissue as compared with subcutaneous adipose tissue human adipose tissue, this fact could provide a novel mechanism by which visceral fat accumulation can promote the development of Type 2 Diabetes Mellitus (**Sethi et al., 2005**).

.

Aim of work

The aim of the work is to study the role of plasma Visfatin in Obesity and type 2 DM.

Obesity

Definition:

In its most simplistic definition, obesity means an excess body fat. Excess weight per se, is an inadequate definition because weight can increase as a result of increased mass, fluid retention, pregnancy leading to a state of overweight in which weight exceeds a standard based on height. It is possible to be obese at a weight within normal, according to standard tables, just as it possible to be overweight without being obese. However, in most people, overweight and obesity tend to parallel to each other (*Fetissov, 2009*). Obese individuals differ in the amount of excess fat they store, and also in the regional distribution of that fat within the body. The distribution of fat induced by weight gain affect the risk associated with obesity and the kind of the disease that result (*WHO, 2000*).

Measurement:

Overweight and obesity are assessed in a variety of ways depending on the necessity for accuracy.

I-Weight for height: the tables of the Metropolitan Life Insurance Company are widely used to establish a standard of ideal body weight (IBW). The term overweight refers to an excess of body weight and usually determined by comparing a person's weight against the standard height and weight chart. If a person's weight is 10 percent greater than the standard, he or she is considered to be overweight, and obese if the weight is 20 percent or more than the standard weight for height, morbid obesity when one weighs in excess of 30 percent of the standard. A person is underweight if he or she is 10 percent below recommended weight for height (*Fetissov, 2009*).

2- Body mass index (BMI): the more preferred methods include body mass index (BMI), or Quetelet index (W/H^2), the formula used for the BMI was developed over a hundred years ago by a