Relation of Serum Vaspin Level to Atherogenic Risk Factors in Type 2 Diabetic Subjects

Thesis

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Rana Hashim Ibrahim

Contents

List of Abbreviations

ACE2	A ngiotensin converting enzyme-2
ADA	American Diabetes Association
AGEs	advanced glycation end products
ARIC	Atherosclerosis Risk in Communities study
ASC	caspase-recruitment domain
ATF3	Activating transcription factor 3
ATGL	Fatty triglyceride lipase

BMI	body mass index
BP	blood pressure
CAD	Coronary artery disease
CB1	endocannabinoid receptor type 1
CCR2	Chemokine receptor 2
CHD	Coronary heart disease
CNS	Central nervous system
CT	Computed tomography
CV	cardiovascular
CVD	cardiovascular disease
DAMPs	damage-associated molecular patterns
DBP	diastolic blood pressure
DM	Diabetes mellitus
DNL	de novo lipogenesis
FABP	Fatty acid binding protein
FATP	Fatty acid transport protein
FFAs	Free fatty acids
FGF1	fibroblast growth factor 1
FGF21	Fibroblast growth factor 21
GRP 78	Glucose regulated protein 78
GSIS	glucose stimulated insulin secretion
HDL	high density lipoproteins
HECT	hyperinsulinemic-euglycemic clamp technique
hK7	human kallikrein 7

HOMA	H omeostatic model assessment
HOMA-IR	Homeostatic model for insulin resistance
HPA	H ypothalamo-pituitary-adrenal
hsCRP	High-sensitivity C-reactive protein
HSL	Hormone sensitive lipase
HT	Hypertensive patients
ICAM-1	I ntracellular cell adhesion molecule-1
i.c.v	Intracerebrovascular
IFN-γ	Interferon-γ
IGF-1	I nsulin-like growth factor-1
IL-6	Interlukin-6
IMT	Intema-media thickness
IRS-2	I nsulin receptor substrate 2
Jmjd3	J umonji domain containing-3
JNK	c-Jun NH2-terminal kinase
LDL	L ow density lipoproteins
LMIC	Low and middle income countries
LPL	Lipoprotein lipase
MCP-1	Monocyte chemoattractant protein-1
MC4R	M elanocortin-4 receptor
MENA	Middle East and North Afreca
Met.S	Metabolic syndrome
MODY	Maturity onset diabetes mellitus

MPs	Microparticles
MRI	Magnetic resonance imaging
MS	Metabolic syndrome
MTP	M icrosomal triglyceride transfer protein
NAD	N icotinamide adenine dinucleotide
NAFLD	N onalcoholic fatty liver disease
Nampt	N icotinamide phosphoribosyltransferase
NASH	Non-alcoholic steatohepatitis
NEFA	Nonesterified fatty acid
NGT	Normal glucose tolerance
NHANES	National Health and Nutrition Examination Survey
NF-κB	Nuclear factor – Kb
NIH	National institutes of health
NMN	N icotinamide mononucleotide
NO	Nitric oxide
NOD	N ucleotide-binding oligomerization domain
NT	Normal tensive
OLETF rats	Otsuka Long-Evans Tokushima Fatty rats
PCOs	Polycystic ovary syndrome
PAD	Peripheral arterial disease
PAI-1	plasminogen activator inhibitor-1
PK C	Protein Kinase C
PPARγ	peroxisome proliferator-activated receptor γ
PUFA	Polyunsaturated fatty acid

RAS	Renin–angiotensin system
RBP-4	Retinol-binding protein-4
ROS	reactive oxygen species
SAT	Subcutaneous adipose tissue
SC	Subcutaneous
SOCS-3	Suppressor of cytokine signaling-3
SVF	Stromal vascular fraction
TIA	Transient ischemic attack
TLR4	Toll-like receptor 4
TNF-α	Tumour necrosis factor-α
TZDS	Thiazolidienamide
VAT	Visceral adipose tissue
VCAM-1	V ascular cell adhesion molecule-1
VLDL-C	Very low density lipoprotein-cholesterol
vWF	v on Willebrand factor
WAT	White adipose tissue
WC	Waist circumference
WHR	Waist over hip ratio

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Introduction

Type 2 diabetes mellitus (DM) is a chronic metabolic disorder in which prevalence has been increasing steadily all over the world with the number of people affected expected to double in the next decade due to increase in aging population (Olokoba et al., 2012) .

People living with type 2 DM are more vulnerable to various forms of both short and long-term complications, which often lead to their premature death, this tendency of increased morbidity and mortality is seen in type 2 DM because of the commonness of this type of DM, its insidious onset and late recognition especially in resource-poor developing countries like Africa (Azevedo and Alla., 2008).

Increase weight is an established risk factor for type 2 DM, recent studies have identified links between obesity and type 2 DM involving pro inflammatory cytokines, insulin resistance, deranged fatty acid metabolism and mitochondrial dysfunction (Eckel et al., 2011).

The influence of obesity on type 2 DM risk is determined not only by degree of obesity but also by where fat

accumulate. Increased upper body fat (visceral adiposity) as reflected in increased waist-hip ratio is associated with Metabolic Syndrome, Type 2 DM and Cardiovascular disease(CVD), whether subcutaneous fat lack the pathological effect of visceral fat or not requires further study (Cypess et al., 2009).

Increased visceral adiposity is usually associated with a clustering of atherogenic risk factors, such as insulin resistance, hypertension, dyslipidemia, alterations in coagulation and inflammatory cytokine profiles (Gulcelik et al., 2009).

Adipose tissue secretes several bioactive peptides that excrete paracrine and endocrine effects and play an important role in metabolic regulation, thus dysregulation of adipokine secretion may link obesity to its related metabolic disorders (Wozniak et al., 2009).

Visceral adipose tissue derived serpin (Vaspin) is a noval adipokine with insulin sensitizing effect originally isolated from the visceral adipose tissue of Otsuka long-Evan Tokushima(OLETF) fatty rats, animal model of type 2 DM (Lee et al., 2008).

Plasma Vaspin concentration is markedly elevated in humans with high body mass index (BMI) and correlates negatively with insulin sensitivity, (Kadoglou et al., 2011). The increase in vaspin may be a compensatory response to antagonize the action of other unknown proteases that are up-regulated in obesity and in states of insulin resistance; hence this regulation may be defensive mechanism against insulin resistance (Gulcelik et al., 2009).

Aim of the Work:

The aim of this study is to evaluate serum Vaspin level as related to atherogenic risk factors such as insulin resistance, dyslipidemia, hypertension in obese type 2 diabetic subjects and obese non diabetic subjects.

Obesity

Obesity is a disorder of body composition defined by a relative or absolute excess of body fat and characterized by several remarkable features and the presence of excess body fat usually-but not always-results in higher body weight. (Hellerestien and Parks., 2007).

The prevalence of obesity has increased worldwide and is a source of concern since the negative consequences of obesity start as early as in childhood. The most commonly used anthropometric tool to assess relative weight and classify obesity is the body mass index (BMI). However, BMI cannot make the distinction between an elevated body weight due to high levels of lean vs. fat body mass. Adipose tissue is now considered as a key organ regarding the fate of excess dietary lipids, which may determine whether or not body homeostasis will be maintained (metabolically healthy obesity) or a state of inflammation/insulin resistance will be produced, with deleterious CV consequences. Obesity, particularly visceral obesity, also induces a variety of structural adaptations/alterations in CV structure/function. (Bastien et al., 2014).

x 1. See all References 1 x 2. See all References 2 The American Heart Association and the American College of Cardiology guidelines labeled obesity as a major modifiable cardiovascular disease (CVD) risk factor. Obesity is associated with higher rates of insulin resistance, type 2 diabetes mellitus (DM), hypertension (HTN), dyslipidemia, coronary heart disease (CHD), gallbladder disease, obstructive sleep apnea, non-alcoholic fatty liver disease and some malignancies including endometrial, breast, and colon cancer. x 4. Pi-Sunyer, F.X. The obesity epidemic: pathophysiology and consequences of obesity. Obes Res . 2002; 10: 97S–104S

CrossRef | PubMed See all References Obesity is considered an independent risk factor for CVD and is associated with increased mortality in general healthy populations. (Oliveros et al., 2014).

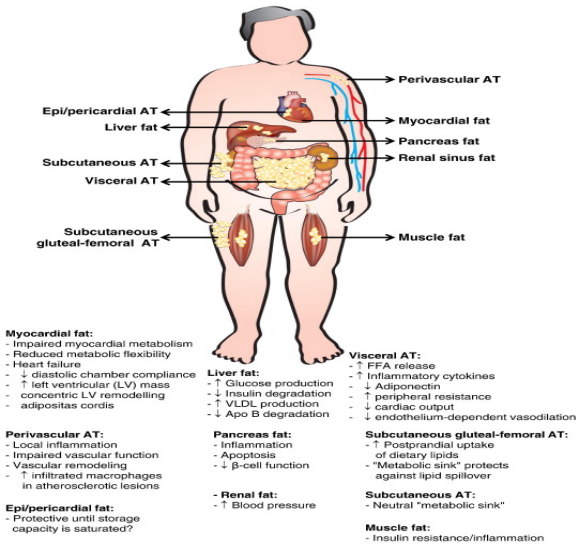
Numerous excellent scientific review papers and academic books have been published on the causes, health consequences, and pathophysiological aspects of obesity . A phenomenon that has received increasing attention is the fact that body shape, and more specifically the regional distribution of adipose tissue, is at least as important, if not more important, than the total amount of body fat in predicting disease-causing complications that have been traditionally associated with obesity. Literature on regional adipose tissue distribution and metabolism has flourished over the past 25 years, establishing beyond any doubt that the proportion of abdominal adipose tissue is a key correlate and perhaps driver of the health risk associated with overweight and obesity. Visceral obesity has now been established as being part of a complex phenotype

including adipose tissue storage dysfunction and ectopic triglyceride accumulation in several sites including the liver . (Despes., 2011).

Fig 2 Excess abdominal visceral adipose tissue, irrespective of the BMI, has been associated with a constellation of diabetogenic and atherogenic abnormalities such as insulin resistance, increased triglycerides and apolipoprotein B levels, low high-density lipoprotein cholesterol and an increased proportion of small dense low-density lipoprotein (LDL) and high-density lipoprotein (HDL) particles, the latter lipid abnormalities being generally described as the atherogenic dyslipidemia Fig 3 . On the contrary, low levels of visceral adipose tissue and subcutaneous obesity are associated with a low risk metabolic risk profile. x 31. Despres, J.P., Moorjani, S., Lupien, P.J., Tremblay, A., Nadeau, A., and Bouchard, C. Regional distribution of body fat, plasma lipoproteins, and cardiovascular disease. Arteriosclerosis . 1990; 10: 497–511

CrossRef | PubMed See all References 31 There is now considerable evidence to support the notion that regional fat accumulation is much more important in CVD risk stratification than excess total adiposity *per se* . (Despres ., 2012).

Fig 1 : Abnormalities increasing risk of cardiovascular disease among overweight/obese individuals with excess vis ceral adipose tissue/ectopic fat. (Bastien et al., 2014).



x 3. Poirier, P., Giles, T.D., Bray, G.A. et al. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. Circulation . 2006; 113: 898–918

CrossRef | PubMed | Scopus (918) See all References 3

Historical Perspective

In 1947, Professor Jean Vague, a French physician from the University of Marseille, reported for the first time in a French medical journal clinical observations that d espite not having access to sophisti cated investigative tools, he identified two different body shapes representing both ends of a spectrum. He coined the term *android obesity* to refer to adipose tissue accumulated preferentially in the trunk/upper body area and suggested that this was a form of obesity closely associated with diabetes and heart disease . He also proposed the term *gynoid obesity* to refer to preferential adipose tissue accumulation in the hips and thighs, typically described as female obesity, a form much less associated with complications. Android and gynoid obesities have also commonly been referred to as “apple and pear shape” obesities, respectively, by the lay press. Vague later summarized his work in the English scientific literature in a paper which was initially received with skepticism by the medical community . Decades later, his seminal early contribution is now finally recognized, as hundreds of studies now support the notion that body fat topography is an important correlate of cardiometabolic health. (Vague., 1956).

In the early 1980s, Per Björntorp, Marcin Krotkiewski, Lars Sjöström, and Ulf Smith from the University of Gothenburg reported in a landmark paper that adipose tissue morphology, body shape, and the regional accumulation of body fat were key factors related to metabolic complications . (Krothiewski et al., 1983).

In 1984, the Gothenburg group also reported results of a prospective study conducted in middle-aged men and women in whom the ratio of abdominal waist over hip circumferences (WHR) was used as a simple index of body fat distribution . They reported that an increased abdominal waistline relative to hip girth was predictive of a higher risk of coronary heart disease (CHD). (Lapidus et al., 1984).

By publishing these observations in the early 1980s, Björntorp and his colleagues sparked the interest of the scientific and medical community for body fat distribution as a clinically relevant phenotype. The mid 1980s should therefore be considered as a “renaissance” period during which regional adipose tissue distribution truly became the focus of interest of several obesity scientists.

For instance, during this period, Ahmed Kissebah and his team from the University of Wisconsin in Milwaukee published several papers that were fully in line with the observations of the Gothenburg group in showing that the proportion of abdominal adipose tissue, crudely estimated by the WHR, was predictive of metabolic abnormalities increasing the risk of both type 2 diabetes and CVD. By 1985, these two groups were conceptually leading the field of obesity on the international scene by providing early evidence that body fat distribution assessed by anthropometry was a critical predictor of metabolic abnormalities. Such early evidence was received with considerable interest, and numerous studies were launched during that period to explore further the contribution of regional adiposity to metabolism and related risk. (Kissebah and Krakower., 1994).

The development of imaging technologies such as computed tomography (CT) and magnetic resonance imaging (MRI) has allowed remarkable advances in the field of body composition/adipose tissue distribution. (Ferland et al., 1989).

With the use of CT, Fujioka et al. were the first in 1987 to provide evidence that a preferential accumulation of visceral adipose tissue could possibly explain the deterioration in glucose and lipid metabolism observed in obese patients. They reported that subjects with large amounts of visceral adipose tissue had higher fasting plasma triglyceride levels and higher plasma glucose responses following an oral glucose challenge than subjects who had the same BMI but a preferential accumulation of abdomi nal subcutaneous adipose tissue. (Fujioka et al., 1987).

Measurements of obesity:

Body mass index

BMI is ratio of total body weight over height squared (kg/m 2).T he National Institutes of Health (NIH) define a normal BMI as 18.5-24.9. Overweight is defined as BMI 25-29.9 . Grade 1 obesity is 30-34.9, grade 2 o besity is 35-39.9, and grade 3 (extreme) obesity is BMI≥40. (Baron 2008).

The American Heart Association has proposed additional obesity subgroups to take into consideration the rapidly expanding subgroup of patients with massive obesity and introduced grade 4 obesity corresponding to a BMI ≥50 kg/m 2 and grade 5 as a BMI ≥60 kg/m 2 Table 1 . (Poirier et al., 2011).

Waist circumference (WC) and waist/hip ratio (WHR)

As that regional fat accumulation is much more important in CVD risk stratification than excess total adiposity *per se* . On that basis, a simple anthropometric index of total adiposity such as the BMI should be refined by measuring additional indices of fat distribution namely WC, WHR or waist-to height ratio to discriminate higher-risk individuals. (Cornier et al., 2011)

Based on expert’s consensus, the World Health Organization has proposed sex-specific cut-off values of WC associated with increased CVD risk: 94 cm in men and 80 cm in women for increased risk, and 102 cm in men and 88 cm in women for substantially increased risk. (WHO ., 2008).

An increase in both WC and WHR predicted an increased risk of CVD in men and women; a 1 cm increase in WC and a 0.01 unit increase in WHR were respectively associated with a 2% increase and 5% increase in risk of future CVD events. (De Koning et al., 2007).

Body composition

Visceral adiposity can be measured accurately by computed tomography, quantitative magnetic resonance

imaging, and with less precision by dual energy x-ray absorptiometry, many other techniques (air displacement plethysmography, bioelectrical impedance, skinfold thickness, X-ray absorptiometry, hydrostatic weighing, etc.) may also be used to assess adiposity and body composition. (liu et al., 2011). x 39. Waist circumference and waist-hip ratio: report of a WHO expert consultation. World Health Organization, Geneva; 2008 (ISBN 978 92 4 150149)

See all References 39

Col lectively, these techniques all o w for the measurement of fat, fat-free mass, bone mineral content, total body water, extracellular water, total adipose tissue and its subdepots (visceral, subcutaneous, and intramuscular) and ectopic fat depots. (Yeong and Dympna., 2008).

Challenging the simplistic concept of obesity as defined by BMI

Firstly, Ruderman et al., x 21. Ruderman, N.B., Schneider, S.H., and Berchtold, P. The “metabolically-obese,” normal-weight individual. Am J Clin Nutr . 1981; 34: 1617–1621

PubMed See all References 21 challenged the notion that standard weight–height tables were the proper way to determine high-risk groups for obesity associated disorders. They observed normal weight individuals suffering from type 2 DM, premature CHD, HTN and hypertriglyceridemia with associated hyperinsulinemia. They pointed out that these abnormalities could not be explained by skinfold thickness or adipose mass and hypothesized that it was due to larger fat cells. (Ruderman et al., 1981).

The identified metabolically obese, normal weight individuals had benefits when they went through programs of energy restriction and weight loss. If patients were challenged to a 4–12 week period of diet and exercise there was metabolic improvement . (Ruderman et al., 1998).

x 22. Ruderman, N., Chisholm, D., Pi-Sunyer, X., and Schneider, S. The metabolically obese, normal-weight individual revisited. Diabetes . 1998; 47: 699–713

CrossRef | PubMed | Scopus (415) See all References 22 Some studies suggested that the main issue to explain the metabolic abnormalities in individuals not particularly overweight was fat distribution. On the basis of these studies, it was proposed a scoring method to identify a metabolically obese normal weight individual. Depending on the presence of associated diseases or biochemical abnormalities related to insulin resistance, individuals would be assigned a score to base the diagnosis of metabolically obese normal weight. All of these mentioned disturbances predispose the individual to suffer from, x 23. Conus, F., Allison, D.B., Rabasa-Lhoret, R. et al. Metabolic and behavioral characteristics of metabolically obese but normal-weight women. J Clin Endocrinol Metab . 2004; 89: 5013–5020

CrossRef | PubMed | Scopus (81) See all References 23 type 2 DM with a three to four-fold time higher risk than control non-obese individuals (Table 1) . (Meigs et al., 2006).

Karelis et al x 25. Dvorak, R.V., DeNino, W.F., Ades, P.A., and Poehlman, E.T. Phenotypic characteristics associated with insulin resistance in metabolically obese but normal-weight young women. Diabetes . 1999; 48: 2210–2214

CrossRef | PubMed | Scopus (126) See all References 25 realized that insulin sensitivity was related to body composition and that body composition could be a major determinant for the metabolic behavior of individuals. In their review Karelis et al x 27. Karelis, A.D., St-Pierre, D.H., Conus, F., Rabasa-Lhoret, R., and Poehlman, E.T. Metabolic and body composition factors in subgroups of obesity: what do we know?. J Clin Endocrinol Metab . 2004; 89: 2569–2575

CrossRef | PubMed | Scopus (288) See all References 27 addressed the differences among the metabolically healthy obese, the metabolically obese normal weight, the individual at risk of obesity and the metabolically healthy i ndividual (Table 2 Table 2) Table 3 . (Karelis et al., 2004).

Metabolically healthy obese subjects when compared with obese insulin resistant adults have a healthier metabolic risk profile and higher disposition index (insulin sensitivity × insulin secretion). These findings challenge the concept that obesity itself produces β-cell dysfunction. Most metabolically obese normal weight subjects can be identified with risk factors in the familial background, birth weight, adult onset weight gain and central adiposity, physical activity status, and the presence of related pathologies. (Succurro et al., 2008). x 28. Succurro, E., Marini, M.A., Frontoni, S. et al. Insulin secretion in metabolically obese, but normal weight,

and in metabolically healthy but obese individuals. Obesity (Silver Spring) . 2008; 16: 1881–1886

CrossRef | PubMed | Scopus (55) See all References 28

Table 1:

Diagnosis of the metabolically abnormal phenotype through cardiometabolic markers. (Karelis et al., 2004)

Metabolically abnormal: Individu als having ≥2 characteristics; m etabolically healthy: individuals with ≤1 characteristic. *Abbreviations:* HOMA-IR, homeostasis model for insulin resistance; hsCRP, high-sensitivity C-reactive protein

Table 2 :

Differences in metabolic characteristics and body composition in the at risk obese, obese and the metabolically normal individuals . (Karelis et al., 2004).

Metabolically Healthy: ideal metabolic profile and normal weight. “At risk” Obese: increased body fat + abnormal metabolic profile. Metabolically Healthy Obese: Increased fat mass + normal metabolic profile + high levels of insulin sensitivity. Metabolically Obese Normal Weight: normal weight obese individual may or may not have metabolic syndrome and that body fat mass percentage is required to define the term

Prevelance of obesity

Global prevalence:

The prevalence of obesity has increased dramatically worldwide over the last decades and has now reached epidemic proportions. For instance, the global prevalence of obesity has nearly doubled between 1980 and 2008. According to the World Health Organization, 35% of adults worldwide aged overweight in both sexes and 3% for obesity). (Bastien et al., 2014). x 1. Global Health Observatory (GHO): Obesity 2008. World Health Organisation 2013. http://www.who.int/gho/ncd/risk_factors/obesity_text/en/index.html .

See all References 1

In the United States, the prevalence of obesity has increased by 8% between 1976 and 1980, by another 8% between 1988 and 1994 with similar increases between 1988–1994 and 1999–2000. In contrast, data from the last decade (1999–2010) suggest that the prevalence of obesity may have plateaued in the USA. (Flegal et al., 2010).

x 4. Flegal, K.M., Carroll, M.D., Ogden, C.L., and Johnson, C.L. Prevalence and trends in obesity among US adults, 1999–2000. JAMA . 2002; 288: 1723–1727

CrossRef | PubMed See all References According to the latest National Health and Nutrition Examination Survey (NHANES), the age-adjusted obesity prevalence was 35.7% in the United States in 2010 with no sex differences. Extreme obesity has more than doubled since 1988–1994 NHANES, shifting from 2.9 to 6.3% in