

# ABSTRACTS

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## LEVELS OF BIOMARKERS OF ENDOTHELIAL DYSFUNCTION AND CHRONIC INFLAMMATION IN OBESE ADOLESCENTS WITH OR WITHOUT INSULIN RESISTANCE

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### Introductions:

Insulin resistance (IR), endothelial dysfunction and chronic inflammation are three pathological conditions that can co-exist, even if their cause-effect relationship is not yet clarified.

### Objective:

To evaluate the possible relationship between insulin resistance, biomarkers of endothelial dysfunction and chronic inflammation in obese adolescents.

### Methods:

136 obese adolescents and 53 subjects with normal weight were enrolled. The obese subjects were divided into two subgroups according to HOMA: with (n=86) or without IR (n=50). The serum levels of E-selectin (sE), soluble intercellular adhesion molecule 1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), plasminogen activator inhibitor-1 (PAI-1) and plasminogen were measured by ELISA.

### Results:

The obese adolescents with or without IR showed higher levels in insulin and HOMA than controls. The obese with IR also presented higher levels in lipid profile than controls. About biomarkers of endothelial dysfunction and inflammation, increased sE in the obese without IR was the only difference compared with controls. The significant increase in sE, sICAM-1 and PAI-1 was observed in obese with IR. Moreover, the PAI-1 was significantly increased in obese with IR than that in obese without IR. By Pearson analysis, sE, sICAM-1 and PAI-1 positively associated with BMI, HOMA, waist circumference and systolic blood pressure. Furthermore, PAI-1 also positively associated with lipid profile and heart rate in all studied subjects.

### Conclusion:

The obese adolescents with IR demonstrated more chronic inflammation than those without IR, which may contribute

more risks to develop cardiovascular diseases in later life. (HIM2012/002)

## INSULIN VERSUS ORAL HYPOGLYCEMIC AGENT AS INITIAL THERAPY FOR NEWLY DIAGNOSED DIABETES MELLITUS TYPE 2: A SYSTEMATIC REVIEW AND META-ANALYSIS

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### Background and aims:

Several new studies have proposed that oral hypoglycemic agents (OHA) further push pancreatic beta cells to failure. The initial benefits of OHA are caused by increasing insulin secretion from the remaining deteriorating pancreatic beta-cells, most often leading to total failure of beta-cell function. Initial insulin therapy can rapidly address the glucose toxicity and improve beta-cell function in newly diagnosed type 2 diabetics. Hence, it was proposed that there is benefit in initiating therapy with insulin for a few months before maintaining patient on oral hypoglycemic agents. This study aims to evaluate the effectiveness of initial insulin therapy versus oral hypoglycemic agents in terms of glucose control, pancreatic beta-cell function and adverse effects such as hypoglycemia and weight gain.

### Materials and methods:

Four RCTs with newly diagnosed type 2 DM given insulin ( $\pm$  metformin) vs. oral hypoglycemic agent (OHA) (multiple or monotherapy) with outcomes of glycemic control (HbA1c), measures of insulin resistance or beta-cell function, and adverse effects of weight and hypoglycemia were included in this metaanalysis. RCTs with subjects having HbA1C  $\geq$  10%, diabetic emergency, and serious comorbid conditions were excluded.

### Results:

Four studies evaluated the effect of initial insulin treatment on glucose control in term of glycosylated hemoglobin (HbA1c). Three hundred fifteen patients were analyzed-176 received insulin and 139 received OHA. Two studies noted slightly better glycemic control with use of insulin however the other two studies showed an opposite result. In terms of beta-cell function and insulin resistance, two studies with these outcome also gave conflicting results. Presence of substantial heterogeneity prevents us from making a conclusion. Four studies included post treatment BMI as an outcome. Of the 345 patients analyzed, 184 received insulin while 161 received oral hypoglycemic agents. All four studies showed lower post treatment BMI among participants in the insulin treatment arm. An opposite finding was expected as insulin is known to cause weight gain. Main adverse effects were mild and severe hypoglycemic episodes and occurrence of diarrhea.

**Conclusion:**

Among newly diagnosed type 2 DM patients, there is inconclusive evidence that use of insulin compared to oral hypoglycemic agent as initial management resulted in improvement glycemic control, decrease in insulin resistance, and improvement in beta cell function. Heterogeneity of data, short duration of follow-up and lack of clinically relevant outcomes are critical issues that preclude conclusion. Initial insulin therapy does not appear to cause more weight gain than OHA. There also appears to be no associated increase in other adverse effects such as severe hypoglycemic events. The prevalence of diabetes is continually increasing as well as its complications hence a well-designed adequately powered randomized control trial with a longer follow-up and cost-effectivity analysis is recommended.

### PREVALENCE OF METABOLIC SYNDROME AND RISK OF CARDIOVASCULAR DISEASE IN IMPAIRED GLUCOSE TOLERANCE SUBJECTS

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Metabolic Syndrome is the aggregation of conditions that together increases the risk of cardiovascular disease and diabetes mellitus in both normal glucose tolerance (NGT) and impaired glucose tolerance (IGT) subjects. It is estimated that around 20 to 25 per cent of the world's adult population have the metabolic syndrome. Over the last 20 years the prevalence of metabolic syndrome has steadily increased in all populations, and making it one of the major global public health challenge. The objective of this study is to estimate the prevalence of metabolic syndrome and cardiovascular risk factors in impaired glucose tolerance (IGT) subjects.

204 Impaired glucose tolerance and 30 normal glucose tolerance subjects of both genders were selected for the present study according to the American Diabetes Association ADA criteria, on the base of two hour glucose tolerance test. Anthropometric characteristics like Waist circumference, BMI, systolic blood pressure, and diastolic blood pressure were measured with standard techniques. Biochemical parameters like fasting blood sugar, fasting insulin, cholesterol, triglycerides, HDL-C, and LDL-C were determined by standard techniques, the HOMA-IR values were calculated with the help of formula.

It is concluded from the present study that the prevalence of metabolic syndrome is significantly increased according to AACE, ATPIII definition criteria's in impaired glucose

tolerance subject, the study emphasizes strongly that MS is major factor to enhance the incidence of type2 diabetes and cardiovascular diseases in impaired glucose tolerance subjects. It is suggested that preventive measures and treatment can reduce the incidence of CVD and type2 diabetes in our population.

### INSULIN RESISTANCE IN TYPE 2 DIABETES MELLITUS: PROSPECT OF AN UNTOUCHED AREA

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**Background & objectives:**

HOMA estimated insulin resistance is an independent predictor of cardiovascular disease in type-2 diabetic subjects. Lack of exact cutoff value in Indians and the absence of local reference data from Gwalior region of central India for HOMA-IR create a challenging issue for early detection of complications and selecting the treatment option. Our aim was to define a local reference cutoff and its association with various risk variables.

**Methods:**

We randomly selected 50 cases and 50 controls, matched for age and sex, from the teaching hospital of G.R. Medical College Gwalior, India.

**Results:**

Mean HOMA IR for cases was  $4.16 \pm 3.57$  (range 0.22-18.71) while for control subjects was  $2.03 \pm 0.64$  (range 1.08- 4.4). The normal cutoff value was found to be 3.31. A significantly high proportion of the cases (22, 44%;  $p < 0.0001$ ) were above the normal cutoff of 3.31 as compared to controls (2, 4%). HOMA- IR was found to be significantly associated with BMI ( $r=0.41$ ;  $p=0.002$ ), WHR ( $R=0.34$ ;  $P=0.01$ ) and FPI ( $r=0.90$ ;  $p<0.001$ ). Mean of HOMA-IR was significantly higher in Subjects with generalized obesity (without generalized Obesity: cases  $3.04 \pm 1.99$ , control  $2.03 \pm 0.64$ ;  $p<0.0001$  vs. with generalized obesity: cases  $7.7 \pm 5.03$ , control 0) and abdominal obesity (without abdominal obesity: cases  $2.85 \pm 1.5$ , control  $2.03 \pm 0.7$ ;  $p=0.01$  vs. with abdominal obesity cases  $5.69 \pm 4.56$ , controls  $2.05 \pm 0.56$ ;  $p=0.001$ ). Female preponderance for the metabolic syndrome was reported in both cases (female 15, 100%; males 33, 94.28%;  $p>.9$ ) and control (female 14, 93.33%; males 54, 54.28% $p=0.002$ ). Mean of the HOMA-IR was significantly higher in cases with complications like retinopathy ( $9.3 \pm 5.12$ ,  $p<0.01$ ), nephropathy

(7.18±3.29, p<0.01), neuropathy (5.64±2.1, p<0.01), CAD (5.76±0.8, p<0.05) and risk of PVD (5.68±0.1, P<0.0001) as compared to cases without complications.

#### **Interpretation & conclusions:**

We concluded that the cutoff of HOMA-IR was higher in the studied population as compared to reports in other Indian studies, with female preponderance for the metabolic syndrome. Dietary and life style modification could have a positive impact on decreasing the toll of complication in these patients as non obese have less insulin resistance which is significantly associated with complication in this studied population.

#### **RELATIONS OF PARAOXONASE1 55 AND 192 GENE POLYMORPHISMS TO DIABETIC COMPLICATIONS IN AN EGYPTIAN POPULATION**

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#### **Background and aims:**

Type 2 diabetes mellitus is the most common type of diabetes worldwide with serious macro and microvascular complications. It is a polygenic disease characterized by interaction of environmental and genetic factors. The paraoxonase 1 gene (PON1) 55 and 192 polymorphisms have been reported to be associated with type 2 diabetes and its complications. Our aim is to study the relations of the PON1 55 and 192 polymorphisms and enzyme activity to diabetic complications among the type 2 diabetic Egyptian population.

#### **Patients and methods:**

100 type 2 diabetic patients with complications were included; 34 with cardiac complications and 66 with microvascular complications in the form of neuropathy, retinopathy and/or nephropathy, in addition to 100 healthy control subjects of matched age and sex. PON1 55 L/M and 192 Q/R polymorphisms and PON1 enzyme activity serum levels were assessed.

#### **Results:**

The LL genotype of PON1 55 polymorphism and QR and QQ genotypes of PON1 192 polymorphism were more frequent among patients with diabetic complications. The PON1 enzyme activity levels were lower among the diabetic patients than control subjects.

#### **Conclusion:**

PON1 55 and 192 polymorphisms and enzyme activity seem to be related to diabetic complications in Egyptian type 2 diabetic patients.

#### **Key words:**

diabetes, complications, paraoxonase 1, polymorphisms, Egyptian.

#### **EFFECT OF DNA POLYMORPHISMS OF APOLIPOPROTEIN B GENE ON SERUM LIPID PROFILE IN OBESE EGYPTIANS**

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#### **Background:**

Numerous polymorphisms of Apolipoprotein B (ApoB) gene may predispose to obesity, particularly a variable number of tandem repeats (VNTR) polymorphism. association between the 3'APOB-VNTR polymorphism and serum lipid levels was observed in many ethnic groups. Thus, the present study was undertaken to study the association of the 3'VNTR polymorphisms of the ApoB gene in obese Egyptian with normal and abnormal lipid profile.

#### **Methods:**

The 3'APOB-VNTR alleles were determined and classified according to the number of repeats in 180 subjects with simple obesity as well as 100 age and sex matched healthy volunteers. Obese group was further subdivided according to lipid profile into normolipidemic and dyslipidemic groups.

#### **Results:**

Different numbers of alleles were identified in the studied groups. The most frequent allele in all the studied groups was VNTR 31, followed by VNTR 38. VNTR 38 repeats were significantly higher in control group than total obese and Dyslipidemic groups. Dyslipidemia was associated with significantly higher frequency of the homozygous VNTR-SS genotype; while, Normolipidemic group had a significantly lower frequency of the heterozygous VNTR-LS genotype compared to healthy controls.

#### **Conclusions:**

VNTR 38 repeats seems to be protective from lipid abnormality in normal weight and obese. There were high VNTR-SS genotype among subjects with dyslipidemia

indicating that VNTR-SS genotype may be considered as genetic risk factor for lipid abnormality in obese Egyptian.

**Keywords:**

DNA polymorphism - Apoprotein B gene-3'APOB - VNTR polymorphism - obesity- dyslipidemia.

**IDENTIFICATION OF A NOVEL -99A>T IAPP GENE MUTATION IN A NORTH INDIAN TYPE-2-DIABETES PATIENT WITH HYPERTENSION**

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**Aim:**

Several studies conducted worldwide supports that mutations in activator domains of promoter region (-91 to -222 bp) of *IAPP* gene can lead to increased Islet amyloid deposition,  $\beta$  cells destruction and insulin resistance. Considering it a pilot study was conducted to identify amylin promoter mutation and its association in patients diagnosed having both type-2- diabetes and hypertension.

**Methods:**

Fifty Hypertensive type-2-diabetic individuals who are free from any micro-vascular complications, in order to exclude nephropathy induced hypertension and 50 healthy controls were included in our study. Genomic DNA of these individuals were isolated from whole blood, proximal promoter of the amylin gene including 207 bp upstream of TATA box was amplified by PCR, and subject to SSCP analysis. Samples with mobility shifts were selected for genotyping by DNA sequencing.

**Results:**

Strikingly we identified a novel -99A>T mutation in a 35 year old female patient with BMI of 26.4 kg/m<sup>2</sup> and family history of diabetes and hypertension. To elucidate whether the identified mutation disrupts the binding site for transcription factors, potential binding sites in the vicinity of this mutation was screened for using the TESS master (*Transcription Element Search System*) a computer program on TRANSFAC, EMBL, CBIL databases. This -99A>T mutation produced a sequence 5'-ATTGG-3' (corresponding to -101 to -97 of *IAPP* gene promoter) and its complementary sequence 3'-TAACC-5' formed a putative binding site for CAAT box binding transcription factors (CTF) like CBP, CP-1, C/EBP $\alpha$ . All these CTFs are well established transcription activators, their role in initiation

and maintaining efficiency of eukaryotic transcription is also very well established.

**Conclusion:**

This activator domain -99A>T mutation of *IAPP* gene can possibly increase gene transcription, production, deposition of Islet amyloid ultimately leading to pathogenesis of type-2-diabetes.

**TO ESTABLISH AN ASSOCIATION BETWEEN AMYLIN PROMOTER MUTATION -132G>A AND HYPERTENSION ASSOCIATED WITH TYPE 2 DIABETES MELLITUS IN NORTH-WEST INDIAN POPULATION**

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**Aim / Hypothesis:**

The promoter mutation -132G>A which was studied to be present in activator domain of the *IAPP* (Amylin) gene promoter was found to play a role in hypertension associated with type 2 diabetes in different ethnic groups. India being the diabetic capital of the world, this study was conducted to identify the frequency of this particular mutation and its association in patients diagnosed having both type 2 diabetes and hypertension.

**Methods:**

Fifty Hypertensive type 2 diabetic individuals devoid of micro-vascular complications and 50 healthy controls were included in our study. The proximal promoter of the amylin gene including 207 bp upstream of TATA box was amplified by polymerase chain reaction, and subject to Restriction fragment length polymorphism analysis using MwoI restriction enzyme.

**Results:**

The RFLP analysis did not identify any -132G>A mutation neither in cases nor in healthy controls.

**Conclusion:**

The amylin gene mutation -132G>A was found absent in studied North-west Indian population.

**Key words:**

Amylin gene; hypertension; *IAPP* (Islet Amyloid Polypeptide); mutation; RFLP, (Restriction fragment length polymorphism); TATA box; Type 2 diabetes mellitus.

## MULTISLICE COMPUTED TOMOGRAPHY IN STRATIFICATION OF PATIENTS WITH CARDIOVASCULAR RISK FACTORS

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Purpose of this work was study results of the application of cardiac multislice computed tomography (MSCT) program to stratify of patients with cardiovascular risk factors (CVRF).

### Material and methods:

95 patients (76 m /19 w) with CVRF were examined in department of MSCT on device Brilliance (Phillips, Netherlands).

### Results:

All patients were distributed on calcium Score (CC) in Hounsfield Units (HU). CC= 0 HU is detected in 8 patients (0,8%); 0 - 10 HU- in 25 (26,3%); 10 -100 HU- 19 (20%); 100-400 HU-in 22 (23,1%) and 400 and > HU- 21 (22.1%) of patients. According to the MSCT results 56 patients (59 %) had not recommended of invasive coronary angiography (1 group). The rest of the 39 patients (41%) recommended a invasive diagnostics (group 2). Patients of the 2nd group compared with patients of the 1st group were on average older (61, 2 years vs. 54, 9 years), among them there were more men, marked by a higher BMI (29,1 vs. 26.9 kg/m<sup>2</sup>) . Patients had a greater number of CVRF (2, 6) than patients of 1group (1, 8) . Diabetes mellitus (60% vs. 18.9%) and arterial hypertension (55, 9% and 14.7%, respectively) were more frequent in group 2 . The frequency of other CVRF was the following: high level cholesterols (34, 7 vs. 34%), smoking (15,6 vs. 16.3%)/. The average CC was significantly higher (324, 9 vs. 31.7 HU) in 2nd group.

### Conclusion:

Our data confirm that the application of cardiac multislice computed tomography program is important to stratify of patients with cardiovascular risk factors.

## NARINGIN AMELIORATES MYOCARDIAL INJURY AND DYSFUNCTION IN VIVO AFTER ISCHEMIA/REPERFUSION THROUGH REGULATION OF HSP 27 AND 70, PAKT/PENOS AND MAPKS

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### Introduction:

Herein, we postulated that naringin, a widely distributed bioflavonoid and polyphenolic compound predominantly found in grapefruits and related citrus herbs species, owing to its strong antioxidant and anti-apoptotic potential, could be a valuable molecule in alleviating myocardial ischemia–reperfusion (IR) injury.

### Methods:

To evaluate this hypothesis, naringin (20–80 mg/kg/day, p.o.) or saline were administered to rats for 14 days, and on 15th day, one-stage ligation of left anterior descending coronary artery for 45 min was performed, followed by 60 min reperfusion.

### Results:

We concluded that naringin (40 and 80 mg/kg/day, p.o.) not only significantly decreased infarct size, but also improved left ventricular functions and the overall hemodynamic status of the myocardium. This amelioration of post-IR-associated cardiac injury by naringin, were accompanied with increased phosphorylation of Akt/eNOS/Erk and elevated protein expressions of  $\beta$ -catenin, Hsp27 and Hsp70 and. In addition, IR-induced TNF- $\alpha$ /IKK- $\beta$ /NF- $\kappa$ B upregulation and JNK phosphorylation were attenuated by naringin. Moreover, naringin showed anti-apoptotic potential as it upregulated Bcl-2 expression and downregulated caspase-3 expression and TUNEL positivity.

### Conclusions:

Taken together, these results provide convincing evidence of naringin as an invaluable molecule in myocardial IR setting probably due to its fortified antioxidant and anti-apoptotic potential.

## CNX-012-CPD, A DIRECT AMPK ACTIVATOR PROVIDES STRONG GLYCEMIC AND LIPID CONTROL IN BOTH *IN VITRO* AND *IN VIVO* ANIMAL MODELS.

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### Objectives:

AMPK Kinase regulates the coordination of anabolic and catabolic processes and can be an attractive therapeutic target to treat T2DM. We report the anti-hyperglycemic and anti-hyperlipidemic effects of CNX-012-CPD, orally bioavailable small molecule direct activator of AMPK in multiple disease animal models.

**Method:**

Male C57BL/6 mice fed with high fat diet (HFD) were assigned to either vehicle or CNX-012-125 (2.5mg/kg, p.o OD) for 6 weeks. Male golden Syrian hamsters fed with either high cholesterol diet (HCD) alone or with CNX-012-CPD (10mg/kg, p.o. OD) for 8 weeks.

**Result:**

CNX-012-CPD is a highly potent and orally bioavailable compound. It significantly inhibits adipose lipolysis by ~40% and gluconeogenesis by ~60% in rat hepatocytes. CNX-012-CPD reduced fasting blood glucose levels by ~15% (170±6 in HFD Vs 152±9 mg/dl in treatment) and body weight by ~13% (31.85±0.64 in HFD Vs 27±1.16 g in treatment). CNX-012-CPD significantly reduced fasting serum triglycerides by ~27% (189±9 in HFD Vs 134±18 mg/dl in treatment) and a 22% reduction in glucose excursion after the oral glucose challenge (OGTT). CNX-012-CPD showed a 27% reduction in serum cholesterol levels (176±13.28 in HCD Vs 139± 9.78 mg/dl in treatment), 25% reduction in LDL (54 ± 6.46 in HCD Vs 40 ± 4.2 mg/dl in treatment) and 48% reduction in TG (153± 20.68 in HCD Vs 79± 10.25 mg/dl in treatment).

**Conclusions:**

CNX-012-CPD has a potential to provide glycemic and lipid control and also reduce body weight gain and can be good therapeutic agent for the treatment of type 2 Diabetes and dyslipidemia.

### IL-18 ADMINISTRATION CAUSES PROGRESSION OF ATHEROSCLEROTIC LESIONS VIA INDUCTION OF CD36 AND MMP-9 EXPRESSION IN APO E-/- MICE

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**Introduction:**

IL-18 is an inflammatory cytokine with pleotropic action. In the present study we have investigated whether IL-18 is involved in cholesterol efflux and plaque instability.

**Results:**

Three groups of normal chow-diet-fed, male Apo E-/- mice, aged 12 weeks (n=5/group) were employed. Gp I was administered Phosphate buffer saline (PBS) (2 months); Gp II was injected with rIL-18 (30 ng/g/day) (1 month) followed by PBS (1 month). Gp III was treated with rIL-18 (1 month) followed by pyrrolidine dithiocarbamate (PDTC) (10 mg/kg body weight/day) (1 month). Significantly augmented expression of IL-18R $\alpha$  by FACS and plasma IL-18 was observed in Gp II. IL-18 treatment caused significant increase in TC and LDL-C whereas HDL-C was significantly decreased. However, this pattern was reversed in Gp III. In heart and aortic tissues, significantly augmented mRNA expression of IL-18, CD36, MMP-9, NF- $\kappa$ B except LXR- $\alpha$  gene which was significantly reduced was observed in Gp II. rIL-18 treatment significantly increased frequency and lesion area of atherosclerotic lesions in Gp II vs Gp I. Lesion area was significantly decreased in Gp III vs Gp II.

**Discussion:**

Our study reports that IL-18 administration initiates inflammatory cascade by binding with IL-18 R $\alpha$  via NF- $\kappa$ B which are involved in progression and destabilization of atherosclerotic plaques in Apo E-/- mice.

**Summary/Conclusion:**

Our study strongly implicates IL-18 as a proatherogenic and proinflammatory cytokine. This study also reveals that NF- $\kappa$ B blockade with PDTC, blocks inflammatory process of IL-18 through down-regulation of both IL-18 and IL-18R $\alpha$ , thus restoring plaque stability.

**Keywords:**

Recombinant IL-18; Atherosclerosis; Foam cells; CD36; MMP-9; LXR- $\alpha$ , NF- $\kappa$ B.

### IS INSULIN RESISTANCE A DETERMINANT OF LEVELS OF SERUM CYSTATIN C AS A MARKER OF EARLY NEPHROPATHY IN METABOLIC SYNDROME?

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**Objectives:**

Metabolic syndrome (MS) contributes to the pathogenesis of chronic kidney disease (CKD). The rise in serum creatinine for diagnosis of CKD is too late for an early intervention. The present study was designed to explore if serum

cystatin C can identify an early kidney dysfunction in MS patients and if insulin resistance correlates with serum cystatin C.

**Methods:**

100 MS patients (International Diabetes Federation criteria) with creatinine clearance between 60-89 ml/min/1.73m<sup>2</sup> (Modification of Diet in Renal Disease study equation) subgrouped into normoalbuminuric (n=47) and microalbuminuric (n=53) were taken as cases. 100 healthy age and sex matched subjects with creatinine clearance above 90 ml/min/1.73m<sup>2</sup> (normoalbuminuric) were taken as controls. Insulin resistance was assessed by homeostatic model assessment of insulin resistance (HOMA-IR).

**Results:**

Serum cystatin C levels were higher in cases as compared to controls (1.55±0.47 vs 0.77±0.12 ng/ml, p<0.001). Microalbuminuric MS had higher cystatin C levels as compared to their normoalbuminuric counterparts (1.88±0.41 vs 1.18±0.14 ng/ml, p<0.05). Furthermore, cystatin C levels were higher in normoalbuminuric MS as compared to controls (1.18±0.14 vs 0.769±0.12 ng/ml, p<0.001) indicating that cystatin C is raised even before microalbuminuria appears. Receiver operating characteristic analysis showed that optimum cut off of 0.95ng/ml of cystatin C can differentiate normoalbuminuric MS from normoalbuminuric controls (sensitivity: 91%; specificity: 92%). Serum cystatin C correlated with HOMA-IR (rs=0.850, p<0.001) and increased as a function of MS components (p<0.001).

**Conclusion:**

We conclude that serum cystatin C is an early marker of CKD in MS and HOMA-IR is an important determinant of serum cystatin C levels in MS.

**SEQUENTIAL GEL IN SITU DETECTION OF APOLIPOPROTEIN DISTRIBUTION IN NATIVE LIPOPROTEINS SUBCLASSES: A PILOT STUDY**

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**Background:**

The HDL-C hypothesis has been challenged by recent mendelian randomization studies. Much more information can be obtained by analyzing the individual apolipoproteins across the spectrum of particles, a key new

avenue to understand the role of HDL in diabetes and the metabolic syndrome. There are only a few studies using elaborate MALDI-MS proteomics on HDL purified by ultracentrifugation.

**Aims of this study:**

We have developed a practical method to detect the major apolipoproteins in LDL and HDL subclasses in human serum.

**Methods:**

Serum LDL and HDL subclasses are separated by native gradient gel electrophoresis which is followed by transfer and sequential western blot to detect apolipoproteins AI, AII, B, E, CII and CIII and lp(a). In this way distinct apolipoprotein profiles across HDL subclasses are obtained. In a pilot study we tested our method on purified HDL and LDL as well as in serum from hyperlipidemic patients and gender and age-matched healthy controls. HDL subclasses are also analyzed for comparison and control purposes using the Lipoprint HDL system from Quantimetrix (Redondo Beach, CA).

**Results and conclusions:**

Our method allows for detection of the major apolipoproteins in native HDL subclasses and shows a striking differential distribution in different subjects, independent of the HDL-C. Differences in sLDL, apoE, CII and CIII are striking when hyperlipidemic and control subjects are compared. Our procedure is more practical for routine use than MALDI-MS proteomics on purified HDL by ultracentrifugation, which also has the disadvantage of high ionic strengths that lead to loss of surface proteins.

**EVIDENCE FOR THE PRESENCE OF PON1 ACTIVITY IN SMALL, DENSE LDL**

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**Background:**

PON1 is a promiscuous, cardioprotective lactonase carried by HDL and its activity is lower in diabetes and metabolic syndrome.

**Hypothesis:**

We hypothesized that the presence of active PON1 can be detected in lp (a) and small dense LDL using our method.

**Methods:**

We selected a set of subjects survivors of AMI and age and gender matched controls. Serum lipoproteins are separated by native gradient gel electrophoresis (3-8 % and 4-12%, Biorad, Hercules, CA), and PON1 activity was detected using our new method. To confirm the identity of the bands, we detected total apoB, apoB100, apoE, lp (a) on sequential western blots of the same transferred gels. To rule out possible confounding by VLDL and chylomicrons (TRL), we run serum at 12 h fasting and 1, 2, 3, and 5 h postprandial and processed as above, TRL do not enter the gels. The addition of the PON1 inhibitor 2-hydroxyquinoline (1 mM) abolished the bands.

**Results:**

PON1 activity in the high molecular weight lipoprotein fraction co-localizes with apoB100 and Lp(a) and not apoB48.

**Conclusions:**

Our method allows for the detection of active PON1 in lp(a) confirming and expanding on a recent proteomic study which detected its presence. On the other hand, in some subjects, we detected PON1 activity in LDL, namely in small dense LDL, which is a *novel* finding. We posit that PON1 in sLDL stems from large VLDL, and beget sLDL would keep their PON1 complement when preferentially metabolized by hepatic lipase. Further studies are being conducted to confirm these findings.

**IMPAIRMENT OF LIVER FUNCTION IN TYPE 2 DIABETIC PATIENTS TREATED AT THE DIABETIC CENTER IN TIKUR ANBESSA SPECIALIZED TEACHING HOSPITAL (TASTH), ADDIS ABABA, ETHIOPIA.**

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The liver is an insulin sensitive organ. Insulin resistance recognized as a pathological factor in the development of liver functions impairment and non alcoholic fatty liver disease (NAFLD). Studies on liver functions abnormalities in type 2 diabetic patients in Ethiopia are lacking. This study was planned to determine the liver function abnormalities

in patients with type 2 diabetes mellitus and the factors associated with these biochemical changes. A cross sectional study was conducted on type 2 diabetic patients. Out of 100 randomly selected patients, 80 individuals fulfill the criteria set up for inclusion while 20 were excluded. Analysis was carried out to compare the liver function tests and lipid profiles of the patients with the normal 60 control individuals. Mean values of liver function tests and lipid profiles were significantly higher in patients than in the control (P<0.001). In contrary, total protein and high density lipoprotein concentrations in patients were lower compared to control group (P<0.01). overall, 22 patients (25%) had at least one or more abnormal liver function tests and lipid profiles. 39 patients (48.75%), 62 patients (77.5%), 47 patients (58.75%), 52(65%) patients have abnormal total Cholesterol, LDL, TAG, and HDL respectively. Elevated parameters (abnormalities) were greater among persons with type2 diabetic patients. There is less association between liver function impairments with the anti diabetic drugs the patients are taking at p values <0.05.

**Key Words:**

LFT; Alkaline phosphatase; lipid profile; Aminotransferases (ALT & AST)

**KOLAVIRON, A GARCINIA BIFLAVONOID COMPLEX AMELIORATE HYPERGLYCEMIA-MEDIATED HEPATIC INJURY IN RATS VIA SUPPRESSION OF INFLAMMATORY RESPONSES**

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Chronic inflammation plays a crucial role in hyperglycemia-induced liver injury. Kolaviron (KV) a natural biflavonoid from *Garcinia kola* seeds have been shown to possess anti-inflammatory properties which has not been explored in diabetes. To our knowledge, this is the first study to investigate the effect of KV on pro-inflammatory proteins in the liver of diabetic rats. Diabetes was induced by a single intravenous injection of streptozotocin STZ (50mg/

kg) in male Wistar rats. Oral administration of kolaviron (100mg/kg) to diabetic rats five times a week for six weeks significantly ameliorated hyperglycemia and liver dysfunction. Serum levels of hepatic marker enzymes were significantly reduced in kolaviron treated diabetic rats. Kolaviron prevented diabetes induced increase in the hepatic levels of proinflammatory cytokines interleukin (IL)-1 $\beta$ , IL-6, tumour necrosis factor (TNF)- $\alpha$  and monocyte chemoattractant protein (MCP-1). The result of this study demonstrates that the hepatoprotective effects of kolaviron in diabetic rats may be partly associated with its modulating effect on inflammatory response.

**Keywords:**

Diabetes, hepatic injury, kolaviron, proinflammatory cytokine, chemotactic protein

**NON DIABETIC HYPERGLYCEMIA IS HIGHLY PREVALENT IN HEART FAILURE PATIENTS**

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**Introduction:**

Diabetes mellitus (DM) and chronic heart failure (CHF) frequently coexist, leading to increased morbidity and mortality<sup>1</sup>. We and others have previously shown that CHF is an insulin resistant state. However, the prevalence of non-diabetic hyperglycemia (NDH) in CHF is not known. This study sought to assess the prevalence of NDH in CHF patients.

**Methods:**

503 non-diabetic CHF patients were recruited from hospital wards and heart failure clinics in Tayside. CHF patients were further classified as either heart failure with reduced ejection fraction (HFrEF) based on CHF symptoms and echocardiographic evidence of left ventricular systolic impairment or heart failure with preserved ejection fraction (HFpEF) with a documented CHF admission and evidence of preserved left ventricular systolic function. All CHF patients were symptomatic, requiring the use of loop diuretics. All available clinical history and blood measurements including HbA1c were recorded at the time of enrolment. NDH was defined by the American Diabetes Association (ADA) criteria (HbA1c  $\geq$ 5.7% - 6.4%). CHF patients with a previous diagnosis of DM or with an HbA1c (1c) >6.4% were excluded from the study. All-cause mortality was evaluated using cox regression survival analysis model.

**Results:**

The overall prevalence of NDH in all CHF patients was 73.8% at the time of inclusion, with no significant difference in prevalence between HFrEF vs. HFpEF groups (73.8% vs. 77.5% respectively). NDH-CHF patients had higher BMI (28.6 vs. 26.6 kg/m<sup>2</sup>, P=0.007), larger waist circumference (102.7 vs. 97.4 cms P=0.0006) and waist-hip ratio (0.97 vs. 0.95 cms, P=0.01). CHF patients with NDH were found to have reduced renal function (higher serum creatinine (108.0 vs. 99.2  $\mu$ mol/L, p=0.04), eGFR (51 vs. 54 mL/min, P=0.02). NDH-CHF patients were more likely to have atrial fibrillation (34% vs. 29%, P=0.02). With a median follow-up period of 1.05 years, there was no significant difference in all-cause mortality in controls as compared to NDH-CHF patients.

**Conclusion:**

NDH is highly prevalent among non-diabetic CHF patients, in both reduced and preserved ejection fraction populations. NDH-CHF patients have higher BMI, larger waist circumference, poorer renal function and are more associated with atrial fibrillation. With a limited follow up, we found no significant difference in all-cause mortality between NDH-CHF patients and normoglycaemic CHF patients.

**LIGHT AT NIGHT (LAN) AS A RISK OF CORONARY ARTERY DISEASE (CAD) AND TYPE 2 DIABETES IN ROTATING NIGHT SHIFT NURSING PROFESSIONALS**

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**Background:**

Exposure of Light at night (LAN) and Sleep loss may be a novel risk factor for CAD and type 2 diabetes.

**Objective:**

The aim of the present study was to investigate the effect of LAN as a risk of CAD, type 2 diabetes and metabolic syndrome (MS) in night shift nursing professionals.

**Material & Methods:**

In the present case-control study, we recruited 30 night shift nursing professionals, aged 20-40 years, performed day and night shift duties, were randomly selected from the Trauma Center King George Medical University and 30 age sex matched controls were also recruited in this study.

**Results:**

Data were analysed by unpaired t-test. BMI was higher in cases (23.69+1.96) as compared to controls (21.66+4.04) ( $p<0.005$ ). Insignificant difference ( $p>0.05$ ) found in fasting blood sugar between night workers (78.38 +9.40) and controls (75.14+14.77). Fasting insulin level was significantly increased ( $p<0.05$ ) in night workers (4.05+2.45) than controls (2.75+2.53). Insulin resistance was slightly increased among night workers (0.80+0.50) than controls (0.53+0.51) which was statistically significant ( $p<0.05$ ). Triglycerides was significantly increased in night workers (137.99+51.57) as compared to controls (105.00+67.40) ( $p<0.05$ ). Total cholesterol was slightly higher (210.06 + 44.91) in night workers ( $p<0.05$ ). HDLcholesterol was lower in night workers (40.78 + 10.92) than controls (44.86 + 11.33) but it could not reached at significant level ( $p>0.05$ ).

**Conclusion:**

Night shift work is associated with increased risk of insulin resistance and lipid disturbances (i.e. low HDL-cholesterol and high triglyceride levels) making them more prone for metabolic syndrome, CAD and Type 2 diabetes.

**INITIATION OF INSULIN RESISTANCE IN DIET-INDUCED OBESITY BY ATYPICAL PROTEIN KINASE C DEPENDENT UNCOUPLING OF FOXO1 FROM AKT2. - ATYPICAL PKC DISSOCIATES AKT2 AND FOXO1**

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A large percentage of adults in Western societies have insulin-resistant syndromes of diet-induced obesity (DIO), the metabolic syndrome (MetSyn) and type 2 diabetes mellitus (T2DM). Unfortunately, the critical initiating mechanism underlying insulin resistance in these syndromes is obscure. In studies of DIO as a forerunner of MetSyn and T2DM, a frequently used experimental model is the high-fat-fed (HFF) mouse in which the diet contains 60% of calories from fat. These 60%-HFF mice have been found to have diminished insulin signaling to insulin receptor substrate-1 (IRS-1) and Akt, but not atypical protein kinase C PKC (aPKC), in liver, and to IRS-1, Akt and aPKC in muscle; moreover, increases in hepatic ceramide, which directly activates aPKC, seem to be important; nevertheless, the initial insulin signaling abnormality that impairs glucose

homeostasis and causes insulin resistance in this model remains uncertain. Here, we used HFF mice consuming a “Western” diet containing a moderately increased fat content, viz., 40% of calories from milk fat, to better define the starting point for insulin resistance in HFF/DIO. After 10 weeks of consuming this 40%-HFF diet, these mice were obese (15% weight increase), glucose-intolerant, hyperinsulinemic, and had hypertriglyceridemia, hypercholesterolemia and hepatosteatosis. Interestingly, the activity and activation of Akt2, as well as aPKC, was surprisingly excessive in liver, and, as expected, both Akt2 and aPKC activities and activation were diminished in muscle. In concert with increased hepatic Akt2 activity, phosphorylation of Akt2 substrate, glycogen synthase-3' (GSK3'), was increased. However, in marked contrast, despite elevated hepatic Akt2 activity and GSK3' phosphorylation, FoxO1 phosphorylation, which mediates insulin/Akt2 effects on gluconeogenesis, was diminished. Remarkably, daily administration of two newly developed small molecule inhibitors of aPKC that were used to reduce hepatic aPKC activity to normal levels, concomitantly restored Akt2-dependent FoxO1 phosphorylation and diminished the heightened expression of hepatic gluconeogenic, as well as lipogenic enzymes virtually to normal. As a result of these improvements in hepatic signaling and metabolism, insulin signaling to both Akt2 and aPKC in muscle improved, and HFF-induced clinical abnormalities, viz., weight gain, glucose intolerance, hypertriglyceridemia, hypercholesterolemia and hepatosteatosis, were largely prevented. We conclude that hyperactivation of hepatic aPKC by high-fat-feeding and subsequent uncoupling of FoxO1 from Akt2 is an early key, if not initiating, mechanism for development of insulin resistance in HFF/DIO.

**VENTRICULAR DYSFUNCTIONS IN CHILDREN WITH TYPE 1 DIABETES MELLITUS (T1DM)**

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**Introduction:**

Diabetes mellitus is associated with long-term damage, dysfunction and failure of various organs especially the eyes, kidneys, heart and blood vessels. Abnormalities of LV function primarily reflected a diastolic abnormality which was an early sign of diabetic been shown to precede systolic dysfunction in diabetic cardiomyopathy, and had patients. Echocardiography can be used for the diagnosis of diabetic cardiomyopathy or diabetes-induced myocardial dysfunction. Moreover, tissue Doppler imaging (TDI) had emerged as a new sensitive technique for the evaluation of diastolic function.

**Aim of the work :**

was to detect early left ventricular dysfunctions in children with type 1 diabetes mellitus and their correlation with the glycemetic control of these children.

**Subjects and methods:**

this study included two groups, (Group I) included 46 children who were diagnosed as type 1 diabetic patients, and (Group II) which included 23 apparently healthy, age and sex matched children as a control group. They were subjected to thorough history taking, clinical examination, laboratory investigations including total serum cholesterol and triglycerides. Left ventricular functions were assessed by resting Trans Thoracic Echocardiography (TTE) and Tissue Doppler Imaging (TDI).

**Results:**

there were significant higher diastolic indices by both TTE and TDI in type 1 diabetic children than the control group. Diagnosis of definite left ventricular diastolic dysfunction was detected in 5 (10.9%) diabetic children by TTE and in 7 (15%) diabetic children by TDI. Finally, there were insignificant associations between duration of the disease, hypoglycemic attacks, DKA, systolic and diastolic blood pressures, HbA1c% levels and different echocardiographic, tissue Doppler parameters.

**Conclusion:**

Alteration of myocardial function induced by DM may begin earlier than was generally thought and these changes might be not correlated with duration of diabetes nor glycemetic control. Children and adolescents with T1DM already have significant changes in myocardial diastolic function of the LV and seem to be at risk of developing further cardiac dysfunctions.

**Key words:**

left ventricular dysfunctions, Type 1 diabetes mellitus children, Echocardiography, Tissue doppler

**EPICARDIAL FAT AS EARLY VASCULAR DAMAGE PREDICTOR IN PATIENT WITH METABOLIC SYNDROME**

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**Background:**

Increased visceral adiposity is a new marker of metabolic syndrome, early vascular damage and Non-alcoholic fatty liver disease (NAFLD). Epicardial adipose tissue

is a very important factor in the pathogenesis of coronary atherosclerosis.

**Aim:**

To estimate the correlation between epicardial fat thickness, MS and NAFLD. To find out whether there is any epicardial fat thickness association with a) metabolic and clinical parameters b) early atherosclerotic vascular damage which is connected with carotid intima media thickness.

**Methods:**

Epicardial fat thickness was measured by transthoracic echocardiograph in 50 patients with MS and with clinical, laboratory, ultrasound , histology proven NAFLD (24 men, 49±13 years, BMI 34±5 kg/m<sup>2</sup>, waist circumference 115±11 cm; 26 women, 47±13 years, BMI 35± 3, waist circumference 110±10 cm) and in 20 healthy volunteers without MS and NAFLD (11 men, 50±9 years BMI 23±1 kg/m<sup>2</sup>; waist circumference 100 ±9 cm; 9 women, 49±10 years, BMI 20 ±4, waist circumference 90 ±10 cm ).

**Results:**

Patients with MS and NAFLD had significantly higher epicardial fat thickness than the other healthy group (4.88±2.5 and 2.62±1.7 mm, p=0.01). There was a strong correlation between patients' age, BMI, waist circumference, fasting glucose, IMT and the increased epicardial fat thickness (more than 2.7 mm). These figures were much higher in patients with MS and NAFLD than in patients without. Moreover, patients who had the increased thickness of epicardial fat had the more dangerous type of the atherosclerotic plaques which ulcerated more often (because of proinflammatory cytokines are secreted by epicardial fat tissue). As for the indicator of the diastolic function early/atrial peak flow it was much lower in the group of patients with high epicardial fat thickness. The increased epicardial fat associates with HOMA-IR. The more epicardial fat a patient has the higher IR is (p=0.041, OR1.7, 95% CII.036-3.60).

**Conclusion:**

Patients with MS and NAFLD had higher figures of epicardial fat thickness than the group of patients without. Moreover there is the connection between epicardial fat and the clinical parameters. The increased epicardial fat figures were associated with insulin-resistant, early vascular damage, atherosclerotic plaques, NAFLD. The increased epicardial fat can be easily diagnosed with the help of echocardiography. So it is possible to estimate visceral and cardiac adiposity which means that the better prognosis of cardiovascular risk and NAFLD will be provided.

**Keywords:**

Epicardial fat thickness, metabolic syndrome, NAFLD, carotid intima media thickness

## RISK FACTORS FOR NON-ALCOHOLIC FATTY LIVER DISEASE IN RUSSIAN FEDERATION IN NATIONALWIDE DIREG-L-01903 STUDY

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*Moscow Medical University named by Sechenov*

### Aim:

To assess the risk factors for non-alcoholic fatty liver disease (NAFLD) in Russian Federation in the national population-based DIGER study.

### Methods:

In total of 30 787 primary care patients (56 % females, mean age 47.8±16 yrs) were enrolled into open multicenter national-wide prospective study. Careful clinical examination, serum biochemistry (including ALT, AST,  $\gamma$ -GT, lipid spectrum, glucose and hepatitis screening) and abdominal ultrasound diagnostics with precise liver assessment were performed in 30 754 patients.

### Results:

NAFLD was found in 8215 (27 %) of included patients. Within group with confirmed NAFLD liver steatosis was diagnosed in 80.3 %, steatohepatitis in 16.8 %, and cirrhosis in 2.9 % of patients. Of notice, only in 3.6 % of NAFLD patients (1.0 % in all population) the diagnosis has been established *before* DIREG-L-01903 program initiation, despite regular observations of participants in primary care centers. AST was increased  $\geq 1.5$  N in 2816 (9.2 %), ALT was increased  $\geq 1.5$  N in 3144 (10.2 %) of patients.

In total patients population most frequent associated clinical conditions were arterial hypertension (42 %), dyslipidemia (38%), and abdominal obesity (36 %). In total NAFLD patients population following conditions has been found significantly more frequent: arterial hypertension (70 %), dyslipidemia (76 %) and hypercholesterolemia (69 %),  $p < 0.001$  compared with total population. In patients aged from 18 to 29 years abdominal obesity was identified as risk factor, because in was found in 45 % NAFLD patients in comparison with 14 % of patients without NAFLD,  $p < 0.001$ . The significance of abdominal obesity as NAFLD risk factor is decreased with advanced age due to relatively higher prevalence of obesity in patients without NAFLD aged from 40 to 80 years.

NAFLD was diagnosed in 64.3 % of patients with type I diabetes, 69.8 % patients with type II diabetes, 45.2 % of patients with arterial hypertension, 61.5 % of patients with obesity and in 66.9 % in those with metabolic syndrome.

### Conclusion:

Taking into account high prevalence (27 %) of NAFLD in Russian Federation the attention should be given for NAFLD risk factors such as arterial hypertension, dyslipidemia and hypercholesterolemia in all age groups as well as abdominal obesity in patients younger than 39 years. Metabolic factors clustering might explore an important link between metabolic syndrome and NAFLD.

## ENDOTHELIAL FUNCTION AND PULSE-WAVE ANALYSIS IN PATIENTS WITH METABOLIC SYNDROME

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### Purpose:

To evaluate changes in pulse-wave shape and endothelial function (EF) in patients with Metabolic syndrome treated with ACEi (ramipril) or calcium-channel blockers- (CCB).

### Methods:

Sixty one patients (mean age 59±10 years) with MS were enrolled in the study and than randomized to ACEi or CCB-based regimen therapy. EF (in reactive hyperemia test) and pulse-wave characteristics were measured both before and after 5 weeks of treatment using novel finger photoplethysmographic device *AngioScan-01* and traditional ultrasonographic method. Stiffness index (SI), reflection index (RI), augmentation index (Aix), systolic BP in aorta (SPa), digital pulse amplitude augmentation (by photoplethysmography), and flow-mediated dilation (FMD, by ultrasound) were accessed.

### Results:

In the most of patients before the treatment normal SI, and elevated RI, Aix, Spa, and significantly impaired EF were shown. BP goals ( $< 130$  and  $90$  mmHg) were achieved in all patients/. Decrease in SI ( $p < .05$ ), RI and SPa were revealed in both treatment arms, whereas trends towards Aix decrease and EF improvement were demonstrated only in ramipril-treated patients.

### Conclusions:

Pulse-wave analysis in patients with MS demonstrated pattern of increased vascular stiffness and peripheral vasoconstriction, accompanying by impaired EF. Both ACEi and CCB treatment resulted in central BP and SI decrease, whereas only ACEi use was associated with trends in EF and Aix improvement in short-term follow-up.

**GENETIC POLYMORPHISM OF LIPOPROTEIDLIPASE IN METABOLIC SYNDROME AND NAFLD PATIENTS TREATING WITH SIMVASTATIN AND URSODEOXYCHOLIC ACID**

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**Aim:**

Nonalcoholic fatty liver disease (NAFLD) is a common condition associated with Metabolic Syndrome. Our aim was to determinate groups of high cardiovascular risk and start early hypolipidemic therapy due to genetic polymorphism of lipoproteidlipase - one of the main enzymes of lipid metabolism in in metabolic syndrome and NAFLD patients, treating with combined therapy of statins and ursodeoxycholic acid (UDCA).

**Methods:**

77 patients with MS and clinic, laboratory, ultrasound , histology proven NAFLD and laboratory proven dyslipidemia (24 men,53 women, age 50 years; BMI 32.3±4.5kg/m<sup>2</sup>; waist circumference (WC) 103.9±18.7cm). All patients were insulin resistant. Index QUICKI 0.300±0.016. 35% (n=27) of patients had NASH. Genetic polymorphism of lipid metabolism (genotype determination of N291S lipoproteidlipase) was done to all the patients. Patients were divided into 3 groups: 1) n=22 UDCA15mg/kg; 2) n=27 Simvastatin 40 mg; 3) n=28 combination of UDCA and Simvastatin.

**Results:**

Homozygotic GG-genotype N291S lipoproteidlipase was discovered and associated with high cardiovascular risk. Homozygotic GG-genotype N291S lipoproteidlipase was discovered in 27% of patients and was correlated with BMI, insulin, triglycerides, VLDL (p< 0.05). After 6 month level of glucose, insuline, C-peptide were lowed; Index QUICKI was raised (p< 0.05). In the combined therapy the level of TChol was changed from 238.5 ± 42.4 to 211.9±18.9 mg/dl; triglycerides from 245.2 ±120.6 to 180.7±46.9 mg/dl; LDL from 153.6 ± 39.2 to 132.3±16.6 mg/dl; HDL were raised from 36.3 ± 12.4 to 44.7±10.1 mg/dl (p<0.05). Serum transaminases were not raised during the treatment. In the combined therapy group the hepatic tests were normalized: ASAT lowed from 43.3 ± 27.2 to 30.8 ± 10.6 IU/L, ALAT from 51.7 ± 33.2 to 37.1 ± 11.9 IU/L (p< 0.05).

**Conclusion:**

While discovering GG-genotype N291S lipoproteidlipase it is necessary to start early the combined treatment. The

results of the research demonstrates the efficacy and safety of Simvastatin 40 mg/kg combined with Ursodeoxycholic acid 15mg/kg in treatment of dyslipidemia and NASH in MS.

**INSULIN RESISTANCE AND AGE CONTRIBUTE TO TELOMERE LENGTH ATTRITION**

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The length of telomere (TL) is widely considered as a biomarker for cardiovascular aging and cardiovascular diseases. TL reflects an individual's TL at birth and telomere attrition thereafter. TL decreases over the life and their loss is accelerated by chronic inflammation and oxidative stress. Insulin resistance (IR) relates to the inflammatory and oxidative stress status. Whether TL is affected by IR still remains a challenge.

**Aim:**

To determine the effect of glucose metabolism regulation and IR in particular on telomere length. The study group included 301 subjects mean age 51.3 ±12.3 years, free of known cardiovascular diseases, anti-diabetes, antihypertensive and lipid lowering medications. 70 subjects had type 2 diabetes mellitus. Serum fasting glucose (FG), insulin, glycosylated haemoglobin (Hb1Ac) were determined using routine laboratory methods. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as fasting insulin (mU/ml)xfasting glucose (mmol/l)/22. TL was determined by quantitative polymerase chain reaction.

**Results:**

TL had significant inverse associations with age (r = - 0,286, p = 0,003), FG(r = - 0,203, p = 0,043), insulin (r = - 0,318, p = 0,006), HOMA-IR (r = - 0,341, p = 0,004), Hb1Ac (r = - 0,212, p = 0,031). Through multiple linear regression analysis, TL was found to be independently and negatively associated with age, HOMA-IR, insulin level (see table).

Predictor	β	Standard Error	p
Age	-0,026	0,010	0,015
HOMA-IR	-0,176	0,056	0,027
Insulin	-0,061	0,021	0,004

Hb1Ac	-0, 213	0, 148	0, 155
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In all subjects age, HOMA-IR predicted 24% of the variance in TL (p=0,0001)

### Conclusion:

An inverse relationship between telomere length and insulin resistance strongly suggests a considerable impact of glucose regulation on telomere dynamics. Insulin resistance may be the main targets in preventing accelerating aging in patients without clinical manifestations of cardiovascular diseases.

## ERECTILE DYSFUNCTION IN PATIENTS WITH METABOLIC SYNDROME

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### Introduction:

Erectile function is assessed by endothelium function in vessels and the function of the cavernosus bodies. When metabolic syndrome occurs, the dysfunction of the endothelium begins. So the formation of the molecule NO destroys. This molecule is the key point in the mechanism of erection.

### Aim of the study:

to understand whether there is any connection between erectile dysfunction and metabolic syndrome

### Methods:

21 men were chosen at the age 39-62 years. The average age was 49 years old. All men had metabolic syndrome. To assess the pulse wave angioscan was used. Angioscan-01 assessed the type of the pulse wave, the stiffness index and the function of the endothelium. The capillars of the patient were scanned and the occlusal probe was done. Erectile dysfunction in men was assessed with the help of international index of erectile function (IIEF). All men had erectile dysfunction from mild and moderate stages.

### Results:

the stiffness of the vessels is high in all patients. The pulse wave type A is a dominant wave. Also these patients had the dysfunction of the endothelium during the occlusal probe.

### Conclusion:

The results of the study showed that the patients with metabolic syndrome have the dysfunction of the vessels. This fact is the cause of development of erectile dysfunction. Moreover the erectile dysfunction begins earlier than

metabolic syndrome and so it can be the marker of the developing of the metabolic syndrome.

### Key words:

Erectile dysfunction, endothelium dysfunction, metabolic syndrome, angioscan-01

## ASSOCIATIONS BETWEEN VIGOROUS INTENSITY PHYSICAL ACTIVITY AND C-REACTIVE PROTEIN IN U.S. ADULTS

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### Purpose:

To examine the associations between high sensitivity C-reactive protein (CRP) and vigorous intensity physical activity (VIPA) in a nationally representative sample of U.S. adults.

### Methods:

Sample (n=6,242) included adults (> 20 years of age) that participated in the 1999-2006 National Health and Nutrition Examination Survey. Three categories of reported VIPA participation were created: no VIPA (referent), insufficient VIPA (< 500 MET-min-wk<sup>-1</sup>), and meeting the 2008 Department of Health and Human Services (DHHS) recommendation ( $\geq$  500 MET-min-wk<sup>-1</sup>). The dependent variable was elevated CRP (3<CRP $\leq$ 10mg/L). Logistic regression analysis was used to estimate odds ratios (OR) and 95% confidence intervals (CI) adjusting for age, gender, race, smoking status, low-density lipoprotein cholesterol, diabetes, and waist circumference (WC).

### Results:

Analysis revealed significantly lower odds of having an elevated CRP for subjects reporting insufficient volumes of VIPA (OR 0.72; 95% CI 0.59-0.88, p=0.002) or volumes of VIPA meeting the 2008 DHHS recommendation (OR 0.67; 95% CI 0.53-0.84, p=0.001). This protective inverse association was independent of several metabolic risk factors. These included having an augmented WC, a high LDL-C level, diabetes, and smoking status.

### Conclusions:

In a representative sample of U.S. adults, reporting any VIPA was associated with significantly lower odds of having an elevated CRP level when compared to those

reporting no VIPA. These results suggest an inverse dose-response relationship exists between VIPA and elevated CRP levels. Future studies should examine the associations among objectively measured VIPA, CRP, and other markers of metabolic health.

### THE STUDY OF ENDOTHELIAL DYSFUNCTION AND INOS (C150T) GENE POLYMORPHISM IN METABOLIC SYNDROME

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#### Background:

The metabolic syndrome (MS) consists of a constellation of metabolic abnormalities that confer increased risk of cardiovascular disease (CVD) and diabetes mellitus (DM). MS is due to endothelial dysfunction which is caused by imbalance between NO and Endothelin. The role of genetic predisposition in development of MS has also been proposed.

#### Objective:

To study the markers of endothelial dysfunction (Nitric Oxide and Endothelin) and inter-genotypic variation of the expression of iNOS gene (C150T) among the study subjects.

#### Materials and Methods:

We enrolled 50 diagnosed case of metabolic syndrome as per International Diabetes Federation (IDF) criteria and 50 healthy volunteers as control. NO, Endothelin measurement and PCR-RFLP was done to identify the iNOS gene C150T polymorphism and its effect on NO levels was also studied.

#### Result:

Subjects with MS had lower NO levels ( $15.3 \pm 10.3$  vs  $20.9 \pm 11$   $\mu$ M,  $p = 0.032$ ) and higher endothelin ( $2.68 \pm 1.73$  vs  $1.98 \pm 0.82$  fmol/ml,  $p = 0.011$ ). The frequency of T allele (10% vs 9%) was higher in cases. The production of NO was higher in cases expressing the T allele. Conclusion: Our study demonstrated that iNOS C150T polymorphism affects endothelial functional status by altering serum NO levels and hence it may be considered as a potential genetic marker for MS. This would be beneficial for the prior identification of high risk individuals as well as for the monitoring of the disease status through the use of novel serum markers – NO & endothelin.

#### Biography:

Dr. Ashok Kumar Ahirwar has completed MBBS at age of 27 years from Barkattulla University, Bhopal (MP), India and now doing MD ( post graduate degree ) in Medical Biochemistry from Lady Hardinge Medical College, Delhi University, New Delhi, India. He got best poster award in AMBICON-2012, Bhuvneswar, India. and ICMR New Delhi, thesis grant for his research work.

### DYSLIPIDEMIA AND CORONARY HEART DISEASE MORTALITY (CHDM) THE TOTAL CHOLESTEROL/HDL-C RATIO IS A KEY LIPID MARKER TO MONITOR

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The major focus of dyslipidemia therapy has been on monitoring LDL-C. [Recommendations were based on studies which showed that lowering LDL-C reduced CHDM.] The validity of an LDL-C focus suffers as critical triglyceride-rich atherogenic lipoproteins (very-low-density lipoprotein, very-low-density lipoprotein remnant, and intermediate-density lipoprotein), which are commonly elevated in patients with and without diabetes, are not addressed—nor is the importance of HDL-C recognized.

I reported last year at this conference the results of the twenty-one landmark monotherapy statin studies (*Endocr Pract* 2013;19:12A-13A). When the baseline TC/HDL-C ratio was  $>5$ , there was a significant reduction in CHDM in *eight of nine* studies; when the baseline ratio was  $<5$ , only *three of twelve* studies showed any CHDM reduction. This year I examined the results of *nine additional* landmark studies using non-statin drugs alone or in combination with a statin (see below). Similar to the first report, when the baseline TC/HDL-C ratio was  $>5$ , *three of four* studies showed a significant reduction in CHDM. When the baseline ratio was  $<5$ , however, *none of the five* studies showed a reduction in CHDM. In *five* studies, subgroup analysis confirmed a reduction in CHDM when the baseline triglycerides were  $\geq 200$  mg/dL. The TC/HDL-C ratio before treatment in the ACCORD subgroup was 6.30, resulting in a 31% decrease in CHDM. Pre- and post-LDL-C values in the subgroup were similar to the entire group. These findings provide powerful scientific evidence that the TC/HDL-C ratio was superior to the LDL-C in predicting which patients benefited from fenofibrate therapy in the ACCORD study.

**Summary:**

There is compelling evidence supporting a significant reduction in CHDM with pharmacologic intervention when the baseline TC/HDL-C ratio is > 5; furthermore, there is clearly a critical threshold for the TC/HDL-C ratio (< 5) for which further modulating of any lipid parameters (HDL-C and/or LDL-C) offers little additional CHDM benefit. Insurance underwriters [Cholesterol for Life Insurance Companies] only look at the TC and TC HDL-C ratio in assessing risk. They do not look at LDL-C or triglycerides! The time has come for the National Cholesterol Expert Panel to get on board with the insurance companies and determine CHDM risk based on the TC/HDL-C ratio instead of LDL-C.

Study	N =	TC/HDL Ratio		LDL-C		Non-Statins Studies Alone or (+) Statin	CHDM	
		Pre	Post	Pre	Post			
Pre-TC/HDL Ratio > 5								
1. LRC-CPPT	3,806	290/46	6.30	5.52	214	190	bile resins	19%
2. HHS	4,081	289/47	6.15	4.82	197	174	gemfibrozil	34%
SUBGROUP: TGs > 200 mg/dL								71%
3. BIP	3,090	212/35	6.06	4.96	149	139	bezafibrate	none
SUBGROUP: TGs ≥ 200 mg/dL								40%
4. VA-HIT	2,531	177/32	5.53	5.00	113	113	gemfibrozil	22%
Pre-TC/HDL Ratio ≤ 5								
5. ACCORD	5,518	175/38	4.60	3.66	100	81	fenofibrate	none
SUBGROUP:	--	189/30	6.30	4.53	103	80		31%
6. FIELD	9,795	195/43	4.53	3.72	119	94	fenofibrate	none
SUBGROUP: TGs ≥ 204 mg/dL								27%
7. AIM-HIGH	3,398	142/35	4.06	3.01	74	62	niaspan/ statin	
SUBGROUP: TGs ≥ 200 mg/dL								36%
8. CETP ACS	15,871	146/42	3.48	2.91	76	81	dalcetrapib/ statin	none
9. HPS2-THRIVE	26,673	128/44	2.90	2.57	63	57	tredaptive/ statin	none

### INHIBITION OF SERINE PROTEINASE ACTIVITY IMPROVES INSULIN SENSITIVITY IN HIGH-FAT DIET-FED LOW-DENSITY LIPOPROTEIN RECEPTOR-NULLMICE

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**Objective:**

Increasing evidence suggests that enhanced protease-mediated inflammation promotes insulin resistance and results

in diabetes. The aim of this study is to investigate suppression of serine protease activity improves insulin sensitivity in high fat diet (HFD)-fed-low-density lipoprotein receptor-null (LDLR<sup>-/-</sup>) mice.

**Methods:**

All LDLR<sup>-/-</sup> mice were divided into 3 groups receiving separate treatment for 10 weeks: normal chow diet, HFD, and HFD+AEBSF (Iserine protease inhibitor, 5 mg<sup>-1</sup>kg<sup>-1</sup>day<sup>-1</sup>; IP). Serum levels of total and serine protease activities, tumor necrosis factor-α (TNF-α), adiponectin, fasting glucose and insulin sensitivity index were determined in these mice.

**Results:**

Compared to normal diet controls, LDLR<sup>-/-</sup> mice fed with HFD had increased weight gain, fasting glucose, TNF-α, leptin, resistin, insulin resistance (HOMA-IR), total and serine protease activities. Additionally, HFD-treated-LDLR<sup>-/-</sup> mice had significantly enhanced serum concentrations of TNF-α, leptin, resistin, insulin resistance (HOMA-IR), and decreased adiponectin levels. Presently AEBSF in HFD fed-LDLR<sup>-/-</sup> mice had significantly decreased levels of fasting glucose, TNF-α, leptin, and improved insulin sensitivity by HOMA-IR and intraperitoneal glucose tolerance test (all p<0.05). Moreover, Presently AEBSF in HFD fed-LDLR<sup>-/-</sup> mice recovered insulin receptor α, and phosphorylation of phosphoinositide-dependent protein kinase 1 (PKD1), Akt, and glycogen synthase kinase-3 (GSK3β) in the visceral adipose tissue and enhanced circulating adiponectin levels.

**Conclusions:**

Our findings demonstrate that administration of HFD in LDLR<sup>-/-</sup> mice upregulated serine proteinase activity, and induced type 2 diabetes. However, treatment with serine proteinase inhibitor suppressed systemic inflammation, enhanced circulating adiponectin concentrations, and recovered insulin resistance.

**Keywords:** diabetes, insulin sensitivity, serine protease, AEBSF

### INFLAMMATION-ENDOTHELIAL DYSFUNCTION AND CARDIOVASCULAR RISK IN OBESE CHILDREN WITH METABOLIC SYNDROME

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**Aim:**

Endothelial dysfunction associates to cardiovascular risk and atherogenesis. Conditions of Overweight (OW), Obesity (OB) and/or Metabolic Syndrome (MS) in children may modify endothelial function and inflammation. Aim: to describe profiles of markers for endothelial dysfunction, inflammation and subclinical risk in a spectrum of obese children.

**Methods:**

Cross-sectional study. Elementary school children without metabolic disease were included. Condition of OW and OB were assessed according to the 85 and 95 normal percentiles; MS diagnosis was made based on adjusted NCEPATPIII criteria. Serum levels of nitrite (reflecting nitric oxide production), leptin and Interleukin1- (IL1-), were determined by biochemical method or ELISA assay. Ankle-to-Brachial Index (ABI) and Carotid Intima Media Thickness (CIMT) measurements were acquired through sphygmomanometry and ultrasonographic studies.

**Results:**

Eighty-three children, aged 9-11 years old were grouped as either: Normal Weight (NW), OW or OB; and further sub-divided by the presence of MS. We found higher levels of nitrites and leptin in children with OW and OB, as compared with the NW group. Additionally, higher levels of IL1- and nitrites were observed in children with OW and OB, if accompanied by MS; and lower levels of ABI and higher CIMT measures were documented in children with OB and MS.

**Conclusion:**

The increase in the body weight affects markers of inflammation and adipokines. While OB, when associated to MS, affects endothelial dysfunction, inflammation and subclinical cardiovascular risk. This supports the existence of different risk factors in the pathogenesis and clinical consequences within children obesity.

*Topics In This Abstract:*

Pathophysiology and clinical impact. Basic science aspects of children obesity, inflammation, endothelial dysfunction and cardiovascular disease.

**OXIDATIVE STRESS MODIFIES ANKLE TO-BRACHIAL INDEX, CAROTID ARTERY INTIMA-MEDIA THICKNESS AND PREVALENCE OF NAFLD IN SUBJECTS WITH METABOLIC SYNDROME.**

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**Aim:**

To assess the effect of oxidative stress over prevalence of non-alcoholic fatty liver disease (NAFLD), as well as the association with markers of cardiovascular risk in subjects with metabolic syndrome (MS).

**Methods:**

Sixty six Mexican-mestizo volunteers, with no hepatotoxic factors, attending for regular check up to the Internal Medicine Department and who were diagnosed with MS were recruited in this cross-sectional study. Plasma lipids, cystatin C, glucose and liver function tests were determined. Lipid peroxidation and were evaluated through malondialdehyde (MDA) plasma concentration. Clinical work up included ultrasound scanning to detect NAFLD; Ankle-to-Brachial Index (ABI) was considered to reflect endothelial dysfunction, while cardiovascular assessment included ultrasonography to measure carotid artery intima-media thickness (CIMT), as well as calculation of Framingham Cardiac Risk Scale, or the European Cardiovascular Disease Risk (SCORE) to estimate cardiovascular mortality risk.

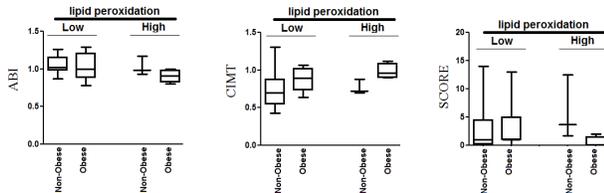
**Results:**

We found that the prevalence of NAFLD co-variated with lipid peroxidation in non-obese subjects; meanwhile, for obese subjects the prevalence of NAFLD co-variated with cystatin C. Interestingly, in subjects with higher levels of lipid peroxidation, the higher body mass index was associated with the worst values of ABI or CIMT; but didn't relate with the estimation of cardiovascular risk.

**Conclusion:**

Our results suggest that oxidative stress and distribution of adipose tissue might regulate subclinical process such as endothelial dysfunction, atherogenesis, as well as NAFLD in subjects with MS.

*TOPICS IN THIS ABSTRACT: Pathophysiology and clinical impact. NAFLD, cardiovascular disease (endothelial dysfunction, atherogenesis) oxidative stress, obesity and metabolic syndrome.*



### ROLE OF DIFFERENT FORMS OF HDL ON OX-LDL INDUCED APOPTOSIS THROUGH AN NF-KBBCL-2 PATHWAY IN DIFFERENTIATED MONOCYTES

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**Objective:**

The simplistic view of atherosclerosis as a disorder of pathological lipid deposition has been redefined by the more complex concept of an ongoing inflammatory response. Macrophages generate a large number of cytokines and growth factors that regulate lesion development. Activation of transcription factor NF-kappaB is an important step in the development of vascular damage, because it controls inducible genes, including many inflammatory mediators. The effect of different forms of HDL on Ox-LDL induced modulation of the expression of NF-kappa B and inducible expression of inflammatory cytokines was studied in view of attenuating atherosclerosis.

**Methods:**

The expression of NF- $\kappa$ B protein and the level of secretory cytokines (TNF- $\alpha$ , IL-8) were measured using specific monoclonal antibodies in Ox-LDL induced differentiated THP-1 cells in presence and absence of different forms of HDL (nHDL; rHDL and Ox-HDL).

**Results:**

An up regulated expression of NF- $\kappa$ B was found in Ox-LDL induced differentiated monocytes and which

was found to be inhibited in the presence of nHDL and rHDL. Further, the increased level of important inflammatory cytokines TNF- $\alpha$  and IL-8 was observed in Ox-LDL induced cells and found to be significantly decreased in presence of nHDL and rHDL.

**Conclusion:**

nHDL and rHDL inhibits NF-kappa B and important inflammatory cytokines TNF- $\alpha$  and IL-8 expression and hence has therapeutic potential for attenuating the progression of atherosclerosis.

### INFLAMMATION, DIABETES MELLITUS AND ESSENTIAL HYPERTENSION

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41 million of the Asian Indian population is diabetic. The prevalence of hypertension is 1.5 to 2.0 times more in the diabetics than in non-diabetic patients. Patients with essential hypertension are more susceptible than normal individuals to develop diabetes. Inflammatory markers such as c-reactive protein (CRP), Interleukin (IL-6) and Tumor necrosis factor (TNF alpha) play a significant role in essential hypertension and diabetes. Present study may help in the better understanding of the correlation of diabetes mellitus and essential hypertension.

**Methodology:**

We have attempted to study various inflammatory markers in whole blood of patients with essential hypertension (N=250), patients with essential hypertension and Diabetes mellitus (N=100) and healthy controls (N=250) in north Indian population such as CRP, IL-6 and TNF alpha by appropriate kits.

**Results and Conclusion:**

In patients with essential hypertension, there was significant increase in levels of CRP (  $p=0.0001$ , 80%), and IL-6 (  $p=0.0001$ , 74%), as compared to normal controls. There was no correlation of TNF alpha with disease essential hypertension. Concomitant presence of diabetes mellitus has not shown any difference in plasma levels of CRP and IL-6 where as significant increase in the levels of TNF alpha. Data suggest a relationship between inflammation and essential hypertension in patients with diabetes, with respect to TNF alpha that has implications for pathogenesis of essential hypertension in the presence of diabetes mellitus.

## INSULIN RESISTANCE EMERGES DURING MULTIMARKER PROGNOSTIC EVALUATION OF HEART FAILURE

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### Background and objective:

Heart failure (HF) present with acute onset dyspnea and has established morbidity and mortality. Identification of high risk patients with insulin resistance for vigilant follow-up using multiple biomarkers strategy in a cohort of acute heart failure (AHF) patients is taken up to identify prognostic significance.

### Methods:

3 month follow-up of 52 consecutive AHF patients with echocardiographic evaluation in NYHA class III/IV enrolled. Nterminal pro-B-type natriuretic peptide (NT-proBNP), high-sensitive cardiac troponin T (hsTropT), high-sensitive C-reactive protein (hsCRP), fasting serum Insulin, ST2 and Uric acid (UA) were evaluated at admission and NT-proBNP, hsTropT repeated 48 hours later. The end-point included cardiovascular death as group 1, re-hospitalisation and refractory HF as group 2.

### Results:

hsTropT and hs-CRP were elevated in 92.3% and 75% patients respectively. Compared to non-ischemic, elevated hsTropT observed in ischemic HF (0.14 ng/ml vs 0.47,  $p < 0.05$ ). A significant correlation between hsTropT, hsCRP, Insulin, glucose, Uric acid & BMI suggests Insulin resistance as a link between inflammation, myocyte injury and oxidative stress in AHF. A rising pattern with worsening renal status was seen group 1 and fall in group 2 for NT-proBNP at 48hrs. A composite-end point was reached in 32.7% of patients during a median follow-up of 4.8 months with an overall mortality of 11.5%. NT-proBNP & ST2 predicted adverse outcomes on follow-up on univariate analysis. 48 hours rise in NT-proBNP pattern ( $>5\%$ ) associated with adverse outcomes; conversely, a fall from baseline in 37% pointed refractory HF.

### Conclusions:

NT-proBNP & ST2 indicated HF. Changing patterns of NT-proBNP predicted adverse outcomes suggesting serial measurement of NT-proBNP. Insulin resistance as a link between inflammation, injury and oxidative stress in AHF may contribute to progressive morbidity.

## Comparison of Groups 1 and 2 with respect to NT proBNP & baseline characteristics

Baseline Characteristic	Group 1	Group 2	p value
Age (in years)	54.1±16.8	59.1±15.7	0.29
% of males	52.9	57.1	0.99
Duration of hospital stay (in days)	5.8±3.1	5.6±2.9	0.82
Diabetes mellitus (%)	47.1	37.1	0.69
Hypertension (%)	35.3	37.1	0.86
Body mass index (kg/m <sup>2</sup> )	23.2±2.6	23.1±2.5	0.89
Blood Glucose (mg/dl)	152.8±119.6	170.9±131.6	0.63
Fasting Serum Insulin	26.72±28.33	27.57±29.73	0.922
IR	10.08±19.08	11.63±22.86	0.999
Hemoglobin (mg/dl)	11.9±2.3	12.8±2.9	0.69
Creatinine (mg/dl)	1.4±0.4	1.3±0.4	0.40
Uric Acid (mg/dl)	8.1±3.3	8.1±3.2	1.00
Sodium (mEq/L)	131.9±6.4	134.7±5.0	0.09
Troponin (ng/ml)	0.28±0.47	0.23±0.61	0.77
hsCRP (mg/dl)	2.2±1.9	2.1±1.8	0.85
LV ejection fraction (%)	37.0±10.1	37.6±9.5	0.84
Adverse events (%)	76.5	11.4	<0.0001*
Mortality (%)	23.5	5.7	0.15

## CONTRIBUTION OF PHYSICAL ACTIVITY TOWARDS TYPE 2 DIABETES AMONG SOUTH ASIANS AND EUROPEANS

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### Background:

Type 2 Diabetes (T2D) is a major risk factor for cardiovascular disease and renal failure, and a leading cause of global morbidity and mortality. South Asians (people from India, Pakistan, Bangladesh and Sri Lanka) are at high risk of T2D. Previous studies show South Asians have lower physical activity levels compared to Europeans. However, contribution of low physical activity levels to high T2D burden in South Asians compared to Europeans remains to be determined.

### Hypothesis/ Aims:

Low levels of physical activity contribute towards the increased risk of T2D among South Asians compared to Europeans. My specific aims are to:

1. Evaluate methods for quantification of physical activity among South Asians and Europeans.
2. Quantify physical activity levels among South Asians and Europeans.

3. Examine the relationship between physical activity and T2D in South Asians and Europeans.

#### Current Progress:

My current focus has been to evaluate the performance of three commercially available accelerometers for the measurement of physical activity and energy expenditure amongst South Asians and Europeans. I recruited 74 participants undertaking an exercise treadmill test (ETT) for clinical indications at Ealing Hospital. All participants fill a questionnaire about personal and medical history, followed by anthropometric measurements (height, weight, waist and hip circumference). The participants then walk on treadmill according to standard clinical protocol while wearing accelerometer devices: one Actiheart (chest position), three Actigraph GT3X devices (wrist, waist and ankle) and three Geneactiv devices (also wrist, waist and ankle). At the end of the test, accelerometer data are downloaded from each device and analysed for energy expenditure (METS) using manufacturer's software. I performed linear regression to quantify the relationship between energy expenditure measured by the accelerometer (Measured) with actual energy expenditure on the treadmill (Expected); the regression co-efficient (beta) provides the estimate of the relationship between Measured and Expected. Measured most closely predicted Expected energy expenditure for ankle-worn devices [beta: Actigraph = 0.55 (0.028); Geneactiv = 0.31 (0.022)] followed by those worn on waist [beta: Actigraph = 0.36 (0.016); Geneactiv = 0.13 (0.008)] and chest [beta = 0.35 (0.021)]. Wrist-worn devices consistently displayed poor prediction of the energy expenditure [beta: Actigraph = 0.008 (0.014); Geneactiv = 0.02 (0.01)]. In both the ankle and waist position the Actigraph GT3X predicted energy expenditure better than the Actiheart or Geneactiv devices. In preliminary univariate analyses, I also found that the relationship of Measured to Expected energy expenditure differed between South Asians and Europeans for both the Actigraph GT3X and Geneactive devices (Heterogeneity  $p < 0.05$ ).

#### Conclusion:

Ankle and waist accelerometers capture energy expenditure more accurately than those on the wrist. The Actigraph GT3X is the most accurate device for measuring physical activity amongst both South Asians and Europeans. However, the relationship between workload measured by devices and the workload predicted by graded exercise test was different between South Asians and Europeans.

#### Future Plan:

These results will be further clarified using multivariate analysis adjusting for different confounding variables that might account for the heterogeneity between the two populations. The device that performs best will be used to quantify physical activity level among South Asians and

determine the contribution of physical inactivity to T2D in South Asians.

#### EARLY INSULIN THERAPY-A NEW TREATMENT APPROACH FOR TYPE-2 DIABETES MELLITUS WITH GLYCOSYLATED HAEMOGLOBIN MORE THAN 7% (HBA1C >7%)

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#### Abstract Summary:

Diabetes is a manageable but serious illness characterized by high blood sugar. According to WHO, 171 million people worldwide suffer from diabetes in the year 2000. In present study comparison of treatment of Metformin, Insulin (Biphasic isophane insulin) and Insulin (Biphasic isophane insulin) plus Metformin in the patients of type 2 diabetes mellitus with glycosylated haemoglobin more than 7% (HbA1c > 7%) was performed. A total 45 patients of type 2 diabetes with HbA1c > 7% were selected. The selected 45 patients were divided into 3 groups of 15 each. First group was treated with Metformin (1000 mg), second group was treated with Insulin (10 IU) and third group was treated with Insulin (10 IU) plus Metformin (500 mg). In the present study an attempt has been made to find out correlation of Insulin, Metformin and Insulin plus Metformin therapy with various parameters like, fasting blood glucose level, postprandial blood glucose level and HbA1c from the data of the patients under study. Duration of treatment was 3 months. Fasting blood sugar (FBS), postprandial blood sugar (PP2BS) and HbA1c level were measured before treatment and after 3 months of each treatment course. Result of this study indicated that significant reduction in fasting and postprandial blood glucose level was observed in each treatment group. In all treatment groups, significant reduction in HbA1c level was also observed. Insulin plus Metformin therapy reduced HbA1c level below 7% while individual Insulin and oral therapy (Metformin) reduced HbA1c near 7%. It is concluded that Insulin (Biphasic isophane insulin) plus Metformin therapy is more effective in the treatment of type 2 diabetes mellitus with HbA1c > 7%. This study provides scientific evidence that insulin therapy in the treatment of type 2 diabetes can be used as first assault rather than last resort.

#### Aim:

Initiation of insulin therapy in type-2 diabetes mellitus improve  $\beta$ -cell function and reduced diabetic complication.

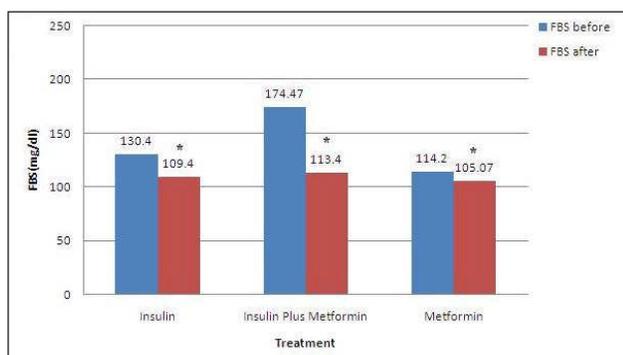
**Result:**

**Table 1.**

Baseline characteristics of the patient population			
	Insulin	Insulin plus Metformin	Metformin
Mean age(years)	61.40	55.20	54.60
Male:Female ratio	10:5	9:6	10:5
Mean weight (kg)	65.40	65.13	60.13
Mean pulse rate (per minute)	76.73	77.13	78.87
Mean systolic pressure (mmHg)	136.40	136.93	135.67
Mean diastolic pressure (mmHg)	79.80	80.67	80.73
Family history of diabetes (%)	86.66	86.66	86.66

**Table 2.**

Effect of Insulin, Insulin plus Metformin, and Metformin treatment on FBS level of type 2 diabetes patients.					
Treatment	N	Dose	Baseline	End of the trial	Mean % change
			Mean ± S.E.M	Mean ± S.E.M	
Insulin	15	10 IU	130.40 ± 3.69	109.40 ± 2.65	16.10
Insulin plus Metformin	15	10 IU, 500mg	174.47 ± 5.89	113.40 ± 2.59	35.00
Metformin	15	1000 mg	114.20 ± 2.47	105.07 ± 2.15	8.00

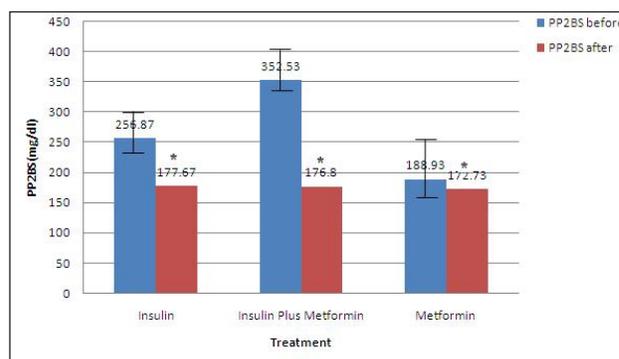


Effect of Insulin, Insulin plus Metformin, and Metformin treatment on FBS level of type 2 diabetes patients. Each bar represents the Mean ± S.E.M (n=15) One way ANOVA

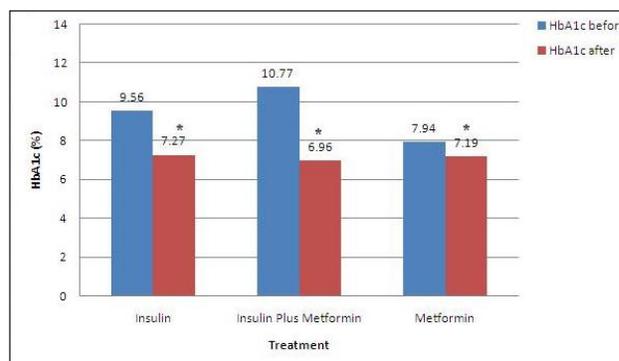
(Analysis of variance), Paired sample t-test \*P< 0.05 when compared with before FBS level.

**Table 3.**

Effect of Insulin, Insulin plus Metformin, and Metformin treatment on PP2BS level of type 2 diabetes patients.					
Treatment	N	Dose	Baseline	End of the trial	Mean % change
			Mean ± S.E.M	Mean ± S.E.M	
Insulin	15	10 IU	256.87 ± 8.82	177.67 ± 5.11	30.83
Insulin plus Metformin	15	10 IU, 500mg	352.53 ± 15.75	176.80 ± 4.60	49.85
Metformin	15	1000mg	188.93 ± 5.35	172.73 ± 4.20	8.57



Effect of Insulin, Insulin plus Metformin, and Metformin treatment on PP2BS level of type 2 diabetes patients. Each bar represents the Mean ± S.E.M (n=15) One way ANOVA (Analysis of variance), Paired sample t-test \*P< 0.05 when compared with before PP2BS level.



Effect of Insulin, Insulin plus Metformin, and Metformin treatment on HbA1c level of type 2 diabetes patients. Each bar represents the Mean ± S.E.M (n=15) One way ANOVA (Analysis of variance), Paired sample t-test \*P< 0.05 when compared with before HbA1c level.

Table 4.

Effect of Insulin, Insulin plus Metformin, and Metformin treatment on HbA1c level of type 2 diabetes patients.					
Treatment	N	Dose	Baseline	End of the trial	Mean % change
			Mean ± S.E.M	Mean ± S.E.M	
Insulin	15	10 IU	9.56 ± 0.19	7.27 ± 0.16	23.99
Insulin plus Metformin	15	10 IU, 500mg	10.77 ± 0.19	6.96 ± 0.15	35.40
Metformin	15	1000mg	7.94 ± 0.16	7.19 ± 0.13	9.40

**Discussion:**

The current paradigm of management of type-2 diabetes is one of sequential addition of treatment modalities starting from medical nutrition therapy, exercise, single or combination oral hypoglycaemic agents (OHAs) and finally insulin administration with or without OHAs. This strategy has miserably failed in achieving recommended glycemic goals to prevent microvascular as well as macrovascular complications. Besides it does not address the fundamental issues of progressive beta cell dysfunction and several other pathogenesis mechanism including first phase insulin response, inflammation, glucotoxicity, lipotoxicity etc.

Insulin administration is uniquely suitable to address most of this issues provided it is started only in the natural history of type-2 diabetes. Short term intensive insulin administration quickly restore normoglycemia provide rest to the stressed beta cells allowing them to regenerate and in maintaining long term glycemic control with diet, exercise and insulin sensitizers.

Insulin is the most effective, yet often underused therapy in type-2 diabetes. Early initiation of therapy to lower HbA1c level is associated with a decrease in the risk of complications.

Early insulin therapy can reduce glucose levels and significant improvement of  $\beta$ -cell function & prevent chronic complication of diabetes in patient of type-2 diabetes having HbA1c > 7%.

After oral antidiabetic drug (OAD) failure, combination insulin and OAD can improve glycemic control with less weight gain than insulin alone in type-2 diabetic patient.

Now withstanding patient's and clinician's reluctance to start insulin at the appropriate time, scientific evidence is loaded in favor of using insulin first assault rather than last resort.

**Conclusion:**

Insulin therapy compared with oral antidiabetic drug (OAD) treatment, could more effectively achieve adequate glycemic control and significant improvement of  $\beta$ -cell function & prevent chronic complication of diabetes in type-2 diabetic patients with severe hyperglycemia.

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**USE OF FOOD LABEL IN CHRONIC DISEASE PATIENTS IN KOREAN ADULTS**

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Korea National Health and Nutritional Examination Survey, 2010-2011 (KNHANES V)*

Nutrition is an important factor of successful chronic disease management. The nutrition label provides a lot of information about nutrition contained in food. However, few studies about association between nutrition label use and chronic disease management. The study was accomplished to investigate the recognition about food-nutrition label in chronic disease patients and how people with chronic disease use nutrition labels to manage their dietary habits, which information is used on nutrition labels. Study based on KNHANES (Korean National Health and Nutrition Examination Survey) data in 2010-2011, multiple logistic regression analysis was used to know the association between nutrition label use and life style modification. Subjects were 11,084, 30.4% were recognized nutrition label. Most interesting factor of label was total energy (46.4%) and 75.3% of using nutrition label subjects were affected by information from label when they purchased of food. Rate of nutrition on patient of chronic disease label was lower than healthy people (9.7% vs 23.7%). Additionally, male nutrition label users were associated with compliance with the dietary reference intake in fiber

(OR 1.97): among female nutrition label users, nutrition label use was associated with compliance with dietary reference intake in sodium (OR 1.19). From these results, the conclusion as follows: Food-nutrient labeling could be useful sources of nutrient information, but patients of chronic disease were not use nutrition label for disease- prevention and life style modification. So, physicians concerned teaching nutrient label for successful chronic disease control and prevention for complication of chronic disease.

**Keyword:** nutrition label, chronic disease, nutrient intake, life style modification

### **ROLE OF ANG(1-7) AND ANGIO II ON THE ANTICONTRACTIL EFFECT OF PERIVASCULAR ADIPOSE TISSUE FROM METABOLIC SYNDROME RATS**

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Perivascular Adipose Tissue(PVAT) exerts a role in vascular tone which consists in releasing relaxing factors like angiotensin(1-7) [Ang(1-7)] that activates Mas receptors and contractile factors like angiotensin II (AngII) which activates AT1 receptors. Ang(1-7) is involved in the anticontractil effect exerted by PVAT. This beneficial effect is attenuated or lost in metabolic syndrome(MetS). The aim of this work is investigate the role of Ang(1-7) and AngII on the anticontractil effect of PVAT in MetS-rats. Wistar rats(3-months-old) were fed with 30%-sucrose in drinking water for 20 weeks. Rats developed hypertriglyceridemia, obesity, hyperinsulinemia and glucose intolerance. Aortic rings were generated and stimulated twice with noradrenaline(NA) through concentration-response curves( $1 \times 10^{-9}M$ - $1 \times 10^{-5}M$ ) with and without A779 or Losartan( $1 \times 10^{-5}M$ ). Furthermore, the release of angiotensins were quantified from Krebs solution by capilar electrophoresis. The anticontractile effect of MetS-group remained at the same magnitude of control-group. Antagonism of Mas receptors produced greater NA responses in +PVAT rings from control-rats, whereas in MetS-rats NA responses were reduced. Moreover, in Krebs solution there was greater release of Ang(1-7) from +PVAT control-rats, but not in MetS-rats. In addition, antagonism of AT1 receptors produced decreased NA responses in +PVAT rings controlrats and also in +PVAT/-PVAT rings MetS-rats. There was greater release of AngII from +PVAT control-rats, but not in MetS-rats. Antagonism of Mas or AT1 receptors increased the release of correspondent endogenous ligand from PVAT in response to NA stimulus,

but this effect is lost in MetS. In conclusion, Ang(1-7) and AngII from PVAT were impaired in MetS leading to vascular dysfunction.

### **THE IMMUNOLOGICAL IDENTIFICATION OF ADIPONECTIN LEVELS ON HUMAN BREAST CANCER TISSUE AND NORMAL MAMMARY GLAND**

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Adiponectin is a peptide hormone secreted from the adipose tissue, affecting the proliferation and insulin sensitivity of various types of cells(2). It is closely and inversely associated with insulin resistance and was recently found to be inversely and independently associated with breast cancer (3). Because insulin resistance in the setting of obesity has also been associated with the development of breast cancer, it may also be operational in the pathogenesis of breast cancer(7,8,9).Epidemiological studies have associated obesity with a range of cancer types, although the mechanism by which obesity induces or promotes tumorigenesis vary by cancer site.(1,4,5,10)These include insulin resistance and resistant chronic hyperinsulinaemia, increased bioavailability of steroid hormones and localized inflammation. In fact, it was found that low serum adiponectin levels are significantly associated with an increased risk for breast cancer and that tumors arising in women with the low serum adiponectin levels are more likely to show a biologically aggressive phenotype(1). The association between obesity and breast cancer risk might be partly explained by adiponectin . In this study, we aimed to compare levels of adiponectin in tissue suspensions in samples from breast cancer and normal tissues.

#### **Material and Methods:**

We included 30 consecutive patients with operable breast cancer and another 30 individuals whose biopsies were benign. All the patients were informed on the study and consented for reserving a part of the biopsy specimen for research purposes. A small part of those biopsies sent to frozen section werecollected and immediately stored at -80 °C until assayed.

After all the specimens were collected, they were weighed by high sensitive pare of scales.They were then homogenized in homogenization solution.Adiponectin were studied in homogenized solutions by ELISA using Human Adiponectin elisa kit (BioVendor Laboratory Medicine, Inc.), respectively. Human Adiponectin ELISA (RD 195023100) is a competetive Enzyme linked

immunosorbent Assay for the quantitative measurement of human adiponectin in serum and plasma. It is intended for in vitro and research use only.

Body mass indexes for all the individuals were calculated. Menopausal status, types of surgery, tumor size, lymph node status, hormonal status, C-erb-B2 overexpression, types of adjuvant chemotherapy and hormonal therapy, radiotherapy were recorded for patients.

Breast cancer was staged in accordance with American Joint Committee for Cancer (AJCC) (September 2003, Journal of Clinical Oncology).

Statistical analyses were performed by using Statistical Package for Sciences.

### Results:

Characteristic of patients and controls are shown in Table 1.

	Breast cancer patients (n=30)	Controls (n=30)
Median age (years)	51.3+ <sub>-</sub> 13	45.4+ <sub>-</sub> 10
Menopausal status	15	15
BMI	25.8+ <sub>-</sub> 2.8	26.6+ <sub>-</sub> 3.9
Type of Surgery	30 case modified radical mastectomy	30 case biopsy
Adiponectin	0.76+ <sub>-</sub> 0.007	0.67+ <sub>-</sub> 0.009

There is no significant difference between the two groups according to age and body mass index ( $p > 0.05$ ).

In both groups of menopausal women, adiponectin levels were found to be high according to nonmenopausal women ( $p = 0.004$ ).

In the group with breast tumor, the median adiponectin level was found 0.76+<sub>-</sub>0.007 and in control group 0.67+<sub>-</sub>0.009. The difference was found to be statistically significant ( $p = 0.001$ ).

In both groups there was no significant difference related with other variables.

### Conclusion:

Future studies are needed to prove causality and provide further insights into both the mechanisms underlying the actions of this hormone and its potential role in breast cancer (6). These results suggest that the low serum adiponectin levels are significantly associated with an increased risk for breast cancer and that tumors arising in women with the low serum adiponectin levels are more likely to

show a biologically aggressive phenotype (1). The association between obesity and breast cancer risk might be partly explained by adiponectin.

In a Harvard case (167)-control (174) study, there was a fairly robust inverse association of adiponectin with breast cancer risk among postmenopausal women (odds ratio, 0.82), no such significant association between adiponectin and breast cancer was found among premenopausal women (8). In our study adiponectin levels in breast cancer tissue is higher than normal mammary tissue. This result shows that the high level of adiponectin levels in mammary tissue is a high risk of breast cancer development.

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### PHYSIOLOGICALLY RELEVANT IN- VITO MODEL OF CHRONIC INSULIN RESISTANCE

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The insulin-signaling network- integral part of metabolic homeostasis regulates blood glucose levels, and when dysregulated, may culminate to type 2 diabetes. Insulin resistance is said to exist whenever normal concentrations of insulin produce a less than normal biologic response. It is intriguing how insulin resistance is developed and how far it deteriorates metabolic signaling of acute insulin. Although numbers of studies have already been reported with *in vitro* hyperinsulinemia IR, most of them have been carried out with acute exposure of non-physiological concentrations (5-100nM). During human hyperinsulinemic euglycemic studies exposure of 500pM of chronic insulin exposure lead to develop IR. Taking clue from these studies, we optimized *in-vitro* conditions wherein differentiated 3T3-L1 adipocyte when exposed to 500 pM Insulin for 72, it leads to IR development. IR was confirmed by reduced phosphorylation of AKT, degradation of IRS, increased leptin secretion and decreased glucose uptake. We also found several defective insulin signaling nodes related to mTOR and MAPK pathways. In this model conditions we have performed concentration dependent (0.1 to 100 nM) temporal signaling events. Therefore understanding of the quantitative relationships between the insulin receptor (IR) and its target activation for mitogenic/metabolic signaling in resistant condition would provide a knowledge base on mechanistic origin of IR, which can be exploited for development of therapeutic interventions in hyperinsulinemia induced pathophysiology.

### PREDICTION OF IMPAIRED GLUCOSE TOLERANCE (IGT) USING THE INSULIN RESISTANCE TEST QUANTOSE™

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#### Objectives:

The oral glucose tolerance test (OGTT) has long been used for the diagnosis of type 2 diabetes and the prediabetic state IGT. Its use has been declining in recent years as it is time consuming, expensive, and unpopular with patients and physicians. A more convenient method for identifying IGT subjects or stratifying risk for IGT could be a useful addition to diabetes risk assessment models. IGT is characterized by insulin resistance (IR) and beta cell dysfunction so it is conceivable that an IR measure could have value in predicting IGT.

#### Subjects and Methods:

The recently developed IR test, Quantose™, was evaluated for its utility in predicting IGT in 4,347 non-diabetic subjects from three observational cohorts: RISC, Botnia, and Mexico City Diabetes. In each cohort, Spearman correlations were generated for the Quantose score, M<sup>Q</sup>, with 2 hour glucose values from the OGTT and AUCs were calculated for ROC curves for predicting IGT using M<sup>Q</sup> alone or in combination with traditional biomarkers of IGT.

#### Results:

Quantose performance was similar in each cohort. Specifically, in the Mexico City Diabetes study, M<sup>Q</sup> correlated with 2 hour glucose values with a correlation coefficient of -0.43 and had an AUC of 0.77 alone and of 0.82 as part of a model incorporating age, sex, BMI, fasting glucose, and triglycerides. In contrast, the same model without M<sup>Q</sup> had a significantly lower AUC of 0.78.

#### Conclusions:

The Quantose score is a powerful predictor of IGT and is complementary with and additive to traditional biomarkers of IGT.

## CONTRIBUTION TO CARDIOVASCULAR REMODELING PROCESS OF TRP64ARG POLYMORPHISM OF BETA-3 ADRENERGIC RECEPTOR GENE

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### Background:

The beta-3 adrenergic receptor gene (ADRB3) is part of the adrenergic system, which is known to play a key role in energy metabolism. Polymorphisms of the ADRB3 have been suggested to participate in the pathogenesis of obesity.

### Objectives:

To study prevalence and contribution to cardiovascular process of Trp64Arg polymorphism of ADRB3 gene in Uzbek hypertensive patients with metabolic syndrome (MetS).

### Design and Methods:

We have examined 169 ethnic Uzbek men with hypertension and MetS. MetS was defined according to IDF, 2005. Genotyping of Trp64Arg polymorphism of ADRB3 gene was determined by RFLP method after PCR amplification and the resulting fragments separated on 3% agarose gels. All patients underwent complete M-mode echocardiography to determine left ventricular mass (LVM). The LVM was indexed to the body surface area to derive the LVM index (LVMI). Flow-mediated endothelium dependent vasodilatation (EDVD) was measured during reactive hyperemia due to 5 minute brachial occlusion. Common carotid intima-media thickness (IMT) was measured by high-resolution ultrasound.

### Results:

The frequency distribution of ADRB3 gene variants and alleles was following: TT genotype – in 75 subjects (44.4%), AT genotype – in 92 ones (54.4%) and AA genotype – in 2 ones (1.2%)  $\chi^2=121.7$ ,  $df=2$ ,  $p=0.000$ , thus Trp64 allele was revealed in 71.6% cases, 64Arg allele – in 28.4% one,  $\chi^2=124.4$ ,  $df=1$ ,  $p=0.000$ . Patients were divided into 2 groups according to carrying of alleles of Trp64Arg polymorphism of ADRB3 gene. The analysis has shown that carriers of Trp allele have had higher levels of SBP and DBP than those with Arg allele. SBP:  $156.4 \pm 16.1$  vs  $151.1 \pm 14.2$  mmHg,  $p=0.0004$ , DBP:  $100.1 \pm 9.8$  vs  $95.8 \pm 7.5$  mmHg,  $p=0.0001$ , respectively. LVM and LVMI were also significantly higher in patients with Trp allele than ones with Arg allele:  $326.8 \pm 80.5$  vs  $302.0 \pm 78.7$  g,  $p=0.011$ , and  $157.2 \pm 38.7$  vs  $145.1 \pm 37.4$  g/m<sup>2</sup>,  $p=0.009$ , respectively. Moreover, in carriers of Trp allele was revealed more impairment of EDVD compared to those with Arg allele:  $\Delta D$   $5.3 \pm 6.2$  vs  $6.9 \pm 5.6\%$ ,  $p=0.002$ . It is necessary

to note, that common carotid IMT was significantly higher in in patients with Trp allele than ones with Arg allele:  $0.93 \pm 0.25$  vs  $0.85 \pm 0.26$  mm,  $p=0.006$ .

### Conclusion:

Results of our study have indicated notable for significantly accumulation AT-genotype and Trp64-allele of Trp64Arg polymorphism of ADRB3 gene in Uzbek hypertensive patients with MetS. Carriage of Trp64-allele of ADRB3 gene contributes to development cardiovascular remodeling in MetS.

## ASSOCIATION BETWEEN COTININE VERIFIED SMOKING STATUS AND METABOLIC SYNDROME IN KOREAN ADULTS: KOREAN NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY

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### Background:

The purpose of this study was to investigate the association between cotinine verified smoking status with metabolic syndrome and metabolic component.

### Method:

11559(5358 males and 6201 females) participants from the Korean National Health and Nutrition Examination Surveys between 2008 and 2010 were recruited. Smoking status was assessed by urinary cotinine and cut-off value for classifying smoker from nonsmoker was 50ng/ml. Presence of Mets was ascertained according to revised National Cholesterol Education Program/Adult Treatment Panel III criteria but for abdominal obesity using definition of Korean Society for the Study of Obesity. Logistic regression analysis was used to evaluate the association between cotinine-verified smoking status and MetS with adjustment for age, body mass index, marital state, education, physical activity, alcohol intake, total fat intake and total fiber intake.

### Result:

Prevalence of Mets was 25.8% (n=2986) ; 26.6 % (n=1427) in men and 25.1 % (n=1559) in women. Risk of Mets was significantly higher in cotinine verified smoker among male( Odds ratio, [OR] = 1.257; 95% confidence interval, [CI]: 1.036-1.525) and female (OR=1.321; 95% CI: 1.007-1.733) in fully adjusted model. For components of MetS, male smoker had an increased risk for high triglyceride (OR=1.299; 95% CI : 1.118-1.511), low high-density

lipoprotein cholesterol (OR=1.395; 1.156-1.684) and decreased risk for high blood pressure (OR=0.808, 95% CI: 0.683-0.956) compared to non-smoker. Female smokers showed increased risk for abdominal obesity (OR=1.519, 95% CI: 1.033-2.234) and high triglyceride (OR=1.449, 95% CI: 1.133-1.853). Furthermore significant positive dose-dependent association between amount of cigarette smoking and risk of metabolic syndrome was observed among male (P for trend=0.009) and female (P for trend=0.021).

**Conclusion:**

This population-based study show that smoking was associated with increased risk for Mets, and this association mainly due to high prevalence of dyslipidemia in male smoker and abdominal obesity in female smoker

**INSULIN RESISTANCE IN WOMEN WITH POLYCYSTIC OVARY SYNDROME: RELATION TO CHRONIC INFLAMMATION AND INTERLEUKIN-6 GENE POLYMORPHISM.**

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*Lady Hardinge Medical College New Delhi*

**Background:**

Polycystic Ovary Syndrome has emerged as the most common endocrinopathy in women of reproductive age group; however its etiology still remains elusive. The discovery of insulin resistance in PCOS along with other metabolic alterations has added new dimensions to its understanding. Low grade systemic inflammation has been implicated to play an integral role in the etiopathogenesis of PCOS by causing insulin resistance. Furthermore, Interleukin-6 (IL-6) gene polymorphisms have been shown to influence insulin sensitivity.

**Methods:**

Serum Insulin, IL-6 and hs-CRP levels were assessed in 50 women with PCOS and 50 healthy age and BMI matched controls by ELISA. DNA extraction, PCR and RFLP analysis were done for studying G174C polymorphism in IL-6 gene.

**Results:**

Mean Insulin, IL-6 and hs-CRP levels were higher in cases as compared to controls. Respective values being (mean cases: mean controls (p value): 16.12µIU/ml: 7.4µIU/ml (< 0.001); 9.59pg/ml: 4.91pg/ml (0.033) and 3.93mg/l:2.49mg/l (0.002). Insulin levels correlated positively with both IL-6 (r = 0.395, p = 0.031) and hs-CRP (r = 0.398, p = 0.029). Significant association was observed between the type of IL-6 polymorphism and serum insulin

levels: the mean value for insulin was 12.6 µIU/ml in C+ (CC and CG) patients (n = 28) and 19.8 µIU/ml in C-(GG) patients (n =22; p =0.048).

**Conclusion:**

Increase in the levels of proinflammatory cytokines causes insulin resistance in PCOS women which at genetic level may be mediated by -174 G/C IL-6 gene polymorphism.

**A NOVEL ANTI-DIABETIC COMPOUND: ABCA1 LIGAND PEPTIDE THAT STIMULATES CELLULAR CHOLESTEROL EFFLUX WITH HIGH EFFICIENCY INCREASES INSULIN SENSITIVITY AND REDUCES BLOOD GLUCOSE IN METABOLIC AND GENETIC MOUSE MODELS OF OBESITY AND DIABETES.**

**John K. Bielicki<sup>1</sup>, Stefanie Bittner<sup>2</sup>, Juveria Tabassum<sup>2</sup>, Anouar Hafiane<sup>3</sup>, Jacques Genest<sup>3</sup>, Salman Azhar<sup>2</sup>, and Jan O. Johansson<sup>4</sup>**

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Cholesterol accumulation in cells has been implicated in the development of diabetes and related events predisposing to atherosclerosis. Recently we developed a family of novel peptides based on the C-terminal (CT) domain of apolipoprotein (apo)E that stimulate cholesterol efflux from cells with high potency and selectivity for ABCA1. Presently we examined whether these peptides improve whole-body insulin sensitivity and lower plasma glucose levels in two mouse models of obesity and diabetes. Two new peptides were identified from preclinical toxicology screens with drastically improved safety margins (NOAEL of 500 mg/kg; i.e. given IV in mice and rats) and greatly reduced TG elevating effects common to other HDL mimetics. These peptides, CS6253 and T6991-2, stimulated ABCA1 cholesterol efflux from macrophages with high efficiency similar to the native apoE CT domain (Km= 0.33±0.14, 0.24±0.02, 0.21±0.02 M, respectively). Administration of CS6253 at a dose of 30 mg/kg (SQ) on alternate days for 6 weeks reduced atherosclerosis by 32% in apoE deficient (apoE<sup>-/-</sup>) mice fed high-fat, high-cholesterol diet (Western diet) for 14 weeks (15±2 vs. 22±4% plaque lesions, CS6253 vs. control, p<0.01). Low-dose (10 mg/kg) administration (SQ) of T6991-2 for 6 weeks in chow-fed *ob/ob* mice showed little effect on steady-state

(basal) glucose levels compared to saline controls (126±22 vs. 135±11 mg/dl, respectively); however, the response following glucose challenge (1 g/kg BW) was greatly reduced with peptide vs. controls (1.8±0.5 vs. 2.8±0.4 fold increases in plasma glucose at 60 min, respectively,  $p<0.01$ ). Treatment of C57BL/6j mice fed high-fat diet (HFD) with T6991-2 (6 weeks, 30 mg/kg) also enhanced insulin sensitivity by 2.2±0.7 fold compared to vehicle-treated controls (55±17 vs. 25±5% reduction in basal glucose levels, respectively, at 15 minutes post insulin, 0.75 Units/kg,  $p<0.01$ ). The favorable anti-diabetic effects of T6991-2 were not associated with any changes in total body weight in *ob/ob* or C57BL/6j mice. Our data suggest HDL mimetic peptides that efficiently target the ABCA1 cholesterol efflux pathway may be useful therapeutically to ameliorate insulin resistance, treat diabetes and promote atherosclerosis regression and plaque stability. These novel observations are of high clinical significance, given that patients with diabetes are at much greater risk of developing heart disease.

#### RELATIONSHIP OF CIRCULATING ENDOTHELIAL AND PROGENITOR CELLS WITH ADIPOSITY AND IMPAIRED FASTING GLUCOSE IN YOUNG POPULATION.

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The obesity and the impaired fasting glucose, dyslipidemia, and elevated blood pressure are risk factors associated to cardiovascular diseases. Circulating endothelial cells (CECs) and endothelial progenitor cells (EPCs) have been considered markers of dysfunction and endothelial damage.

#### Aim:

To evaluate the number of circulating endothelial and progenitor cells with traditional risk factors in youth population with and without obesity.

#### Methods:

We analyzed circulating CEMs and EPCs in peripheral blood obtained from 119 young (18-28 years), 66 with normalweight and 53 with obesity. In all participants were performed anthropometric measurements (Tanita TBF-300). Metabolic parameters were analyzed by colorimetric assay and the quantification of CECs defined as CD146<sup>+</sup>, CD34<sup>+</sup> and CD45<sup>-</sup> cells, and EPCs as CD34<sup>+</sup>, CD133<sup>+</sup> and CD45<sup>-</sup> cells; was carried out by flow cytometry (BD Fasc-Canton II).

#### Results:

The results show that the absolute number of CECs in obese group was higher than normal-weight group ( $p=0.08$ ), whereas for the number of CEPs there was not differences between groups. However, we observed a positive correlation of CECs with EPCs ( $r^*=0.56$ ,  $p<0.001$ ), fat mass ( $r^*=0.20$ ,  $p=0.02$ ), but not with glucose ( $r^*=0.14$ ,  $p=0.11$ ). Also, EPCs correlated with fat-free mass ( $r^*=0.15$ ,  $p=0.09$ ). CECs count was stratified into tertiles, showed association of the upper tertile ( $\geq 7$  cells) with obesity (OR=2.64 (IC<sub>95%</sub> 1.04-6.6),  $p=0.04$ ), and glucose  $\geq 100$  mg/dL (OR=2.62 (IC<sub>95%</sub> 0.4-17.2),  $p=0.31$ ).

#### Conclusions:

The increase in the CECs number was associated with adiposity and impaired glucose, therefore may be considered early markers for cardiovascular disease.

#### Key Words:

Circulating endothelial cells, obesity, impaired glucose, young population.

#### LINK BETWEEN SERUM LEPTIN LEVELS AND INSULIN RESISTANCE IN MEXICAN CHILDREN.

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Leptin is an important regulator of food intake and energy expenditure. The effect of leptin on lipid metabolism, glucose homeostasis and insulin resistance (IR) has been recently summarized.

**Aim:**

To analyze the relationship between leptin levels with metabolic parameters and IR in Mexican children.

**Methods:**

Participants were 174 children (age 6-12 years old). Anthropometric measurement, leptin, insulin and biochemical profile were evaluated. Insulin Resistance (IR) using HOMA (Homeostasis Model Assessment) index was determined considering as the cut-off point the upper percentile 75 (HOMA  $\geq$ 2.4).

**Results:**

Serum leptin levels were correlated with anthropometric measurement: BMI ( $r=0.87$ ), waist circumference ( $r=0.82$ ) and skinfold thickness measurement ( $p<0.001$ ), as well also metabolic parameters: glucose, cholesterol ( $r=0.19$ ,  $p=0.01$ ), triglycerides ( $r=0.49$ ,  $p<0.001$ ), HDL-c ( $r=-0.34$ ,  $p<0.001$ ), insulin ( $r=0.52$ ,  $p<0.001$ ) and HOMA ( $r=0.53$ ,  $p<0.001$ ). Insulin and leptin levels were grouped into tertiles, insulin levels  $\geq 9.5\mu\text{U/mL}$  were associated with glucose  $\geq 100\text{mg/dL}$  (OR=1.50, IC<sub>95%</sub> 0.98-2.28,  $p=0.06$ ), triglycerides  $\geq 150\text{mg/dL}$  (OR=2.36, IC<sub>95%</sub> 1.42-3.91,  $p<0.001$ ), and HDL-c  $<40\text{mg/dL}$  (OR=2.16, IC<sub>95%</sub> 1.36-3.41,  $p<0.001$ ), and leptin levels  $\geq 9.0\text{ ng/mL}$  were associated with glucose  $\geq 100\text{mg/dL}$  (OR=1.39, IC<sub>95%</sub> 0.92-2.10,  $p=0.11$ ), triglycerides  $\geq 150\text{mg/dL}$  (OR=3.72, IC<sub>95%</sub> 2.12-6.53,  $p<0.001$ ), cholesterol  $\geq 200\text{mg/dL}$  (OR=1.64, IC<sub>95%</sub> 1.67-2.5,  $p=0.02$ ), HDL-c  $<40\text{mg/dL}$  (OR=2.67, IC<sub>95%</sub> 1.67-4.26,  $p<0.001$ ) and IR (OR=3.54, IC<sub>95%</sub> 2.17-5.77,  $p<0.001$ ).

**Conclusions:**

Our study showed a link between leptin levels and abnormal metabolic profile and the insulin resistance in the sample children studied.

**Key Words:**

Serum leptin, metabolic abnormalities, insulin resistance, children.

**-572G/C POLYMORPHISM OF IL-6 GENE IS ASSOCIATED WITH INSULINEMIA AND HOMA INDEX IN NORMAL-WEIGHT CHILDREN.**

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The cytokine interleukin 6 (IL-6) is an essential regulator of the acute phase response associated with insulin-resistant states including type 2 diabetes and obesity. Polymorphisms into the IL-6 gene promoter have been associated with the transcription of IL-6 gene.

**Aim:**

To analyze the relationship of SNP -572G/C of IL-6 gene with the insulinemia and HOMA index among Mexican children with obesity or normal-weight.

**Methods:**

A total of 209 children (103 with obesity and 106 with normal-weight), age range of 6 to 12 years were enrolled in the study. Anthropometric measures how weight, height, body circumferences, and skinfold thickness were determined. Serum glucose was determined using a colorimetric assay and insulin levels by ELISA (GenWay). HOMA-IR was calculated as fasting glucose (mg/dL) x fasting insulin ( $\mu\text{U/mL}$ )/405. IL-6 polymorphism was genotyped using the restriction fragment length polymorphism of polymerase chain reaction (RFLP-PCR) method.

**Results:**

Genotype frequencies of SNP in both groups were in the Hardy-Weinberg equilibrium. Normal-weight children, GC carriers showed higher insulin levels (7.41 $\mu\text{U/mL}$ ,  $p=0.002$ ) and HOMA index (1.4,  $p=0.003$ ). In a dominant model, the -572G allele was related with increased of insulin levels ( $p=0.05$ ), waist circumference ( $p=0.07$ ) and suprailiac skinfold ( $p=0.05$ ). The G allele was associated with insulin levels 3rd tertile (OR=2.29; 0.73-7.18;  $p=0.15$ ), insulin resistance (OR=1.79; 0.37-8.7;  $p=0.49$ ) and impaired glucose (OR=2.33; 0.70-7.7;  $p=0.16$ ). The relationship was not found in obese children.

**Conclusion:**

The results suggest that -572G allele of IL-6 gene may be associated with insulin resistance in normal-weight children.

**Key Words:**

IL-6 Polymorphism, Insulinemia, HOMA, children.

## DISTRIBUTION OF FASTING PLASMA INSULIN, GLUCOSE CONCENTRATIONS AND OF HOMEOSTASIS MODEL ASSESSMENT OF INSULIN RESISTANCE IN A REPRESENTATIVE SAMPLE OF TUNISIAN ADOLESCENTS

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### Background:

Plasma fasting insulin and the homeostasis model assessment of insulin resistance (HOMA-IR) are markers of IR, which, at least in part, mediates the relation of obesity to increased cardiovascular risk. Our objective was to describe the distributions of fasting plasma insulin, glucose concentrations and HOMA-IR according to the BMI percentiles in youth.

### Methods:

Fasting plasma insulin and glucose concentrations were measured in a representative sample of Tunisian adolescents comprising 908 individuals. HOMA-IR score was calculated according to the levels of insulin and glucose concentrations.

### Results:

The mean concentrations of insulin was  $50.5 \pm 29.01$  pmol/l among boys and  $62 \pm 37.78$  pmol/l among girls. The distribution of insulin levels ranged increasingly through classes BMI among both girls and boys. The average of the HOMA score was 1.5 for males and 1.81 for females. The HOMA score evolved an increasingly across classes of BMI among both girls and boys. We observed strong correlations between insulin concentrations and HOMA-IR values, as well as close similarity in their rankings of individuals.

### Conclusions:

We report the first data on the distributions of fasting plasma insulin, glucose concentrations and HOMA-IR from a representative sample of youth in Tunisia. These results showed the positive trends of both insulin concentrations and HOMA-IR values according to the BMI percentiles and obesity levels of the studied population.

## HEME OXYGENASE IMPROVES CARDIOMETABOLIC COMPLICATIONS IN OBESITY

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### Abstract:

With the escalation of obesity and related cardiometabolic complications like cardiac dysfunction and type-2 diabetes remains a global challenge. Many obese individuals are normoglycemic and may appear asymptomatic of cardiometabolic complications. However, this apparent state of good health may be a misnomer. We investigated the effects of heme-oxygenase (HO) on inflammation and cardiometabolic complications in normoglycemic obese Zucker-fatty rats (ZFs) given that cardiac complications including heart failure is among the major causes of mortality in obese individuals. Although HO is cytoprotective, its effects on cardiomyopathy in ZFs remain to be elucidated.

The administration of the HO-inducer, hemin attenuated several inflammatory/oxidative mediators including the pro-inflammatory macrophage M1-phenotype, and related inflammatory chemokines/cytokines such as macrophage-chemoattractant protein-1 (MCP-1), macrophage-inflammatory protein-1 alpha (MIP-1), interleukin (IL)-6, IL-1, tumour necrosis factor alpha (TNF- $\alpha$ ), 8-isoprostane and endothelin-1 in ZFs. In contrast, the anti-inflammatory macrophage M2-phenotype, atrial-natriuretic-peptide (ANP), adiponectin, HO-1, HO-activity, cGMP, insulin sensitivity (HOMA-index) and proteins of insulin-signaling including IRS-1, PI3K and GLUT4 were enhanced in ZFs. These were associated with the reduction of cardiac histopathological lesions, cardiomyocyte longitudinal muscle-fiber thickness, cardiomyocyte hypertrophy, fibrosis, extracellular matrix/pro-fibrotic proteins including collagen-IV, fibronectin, TGF-1 as well as the reduction of important markers of heart failure such as osteopontin and osteoprotegerin. Correspondingly, hemin improved important hemodynamic/echocardiographic parameters, including mean arterial pressure, arterial systolic pressure, arterial diastolic pressure, LV-diastolic wall-thickness, LV-systolic wall thickness, LV-developed pressure, +dP/dt and cardiac output. Contrarily, the HO-inhibitor, stannous-mesoporphyrin nullified the hemin effects, exacerbating inflammatory/oxidative insults and aggravated insulin resistance.

These findings suggest that hemin improves cardiac function by reducing osteopontin, osteoprotegerin, pro-inflammatory chemokines/cytokines, macrophage infiltration, macrophage M1-phenotype, LV-hypertrophy, cardiac lesions and extracellular matrix/pro-fibrotic proteins, while potentiating anti-inflammatory macrophage M2-phenotype, insulin-signaling and the HOadiponectin-ANP axis. We conclude that although ZFs are normoglycemic, perturbations in insulin-signaling and cardiac function may be forerunners to overt hyperglycemia and if these problems persist, heart failure may occur.

### **ET-1 CAUSED ADIPOCYTE HYPERPLASIA AND INHIBITED ADIPOCYTE HYPERTROPHY**

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Endothelin-1 (ET-1) is produced dominantly by endothelial cells and functions as a potent vasoconstrictor. Studies also demonstrated that ET-1 affect adipocyte physiology such as ET-1 treatment induced lipolysis in adipocytes and enhanced plasma free fatty acid concentration in rats. Besides, ET-1 has been shown to inhibit adipocyte differentiation in 3T3-L1 adipocytes and human adipocyte precursor cells, but the underlying mechanism is still unclear. In the present study, we conducted in vivo and in vitro studies to explore the effects of ET-1 on adipocyte differentiation and the underlying mechanism. First, male Sprague-Dawley rats were infused ET-1 or saline for four weeks via intraperitoneally implanted osmotic pumps, then the fat pad weight and adipocyte size of epididymal fat were determined. Second, 3T3-L1 preadipocyte cell line was used to explore the effect of ET-1 on cell proliferation and triglyceride (TG) accumulation. The results showed that ET-1 infusion decreased adipocyte size but not epididymal fat pad weight. In 3T3-L1 preadipocyte cell line, we found that ET-1, acting via endothelin A receptor (ETAR), increased cell number during preadipocyte and mitotic clonal expansion stage and caused decrease in TG accumulation and adipogenic transcription factors expression. In addition, ERK pathway was involved in ET-1-inhibited TG accumulation, but not in ET-1-induced preadipocyte proliferation. Taken together, ET-1 induced adipocyte hyperplasia through ERK-independent pathway and inhibited adipocyte hypertrophy partially through ERK pathway.

### **EFFECTS OF EXERCISE TRAINING ON THE MUSCLE MASS, STRENGTH AND QUALITY IN AGED DIABETIC WOMEN**

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#### **Objectives:**

To evaluate the effects of aerobic exercise and resistance exercise on the muscle mass, strength and quality.

#### **Methods:**

We evaluated the impact of a 12-week aerobic exercise or resistance exercise training in aged type 2 diabetic women not on insulin treatment. Thirty six patients had completed the training program. Each subjects randomly allocated to one of three intervention groups, which are aerobic training group (AT=13), resistance training group (RT=13), and no exercise intervention group (CON=10). Body composition was analyzed using bioelectrical impedance analysis and dual energy x-ray absorptiometry. Muscle strength was measured using an isokinetic dynamometer for knee flexion and extension. Muscle quality was assessed by taking the ratio of strength to the entire corresponding leg muscle measured by DXA. Metabolic parameters such as fasting glucose, insulin and hemoglobin A1c level were measured at pre- and post-intervention period

#### **Results:**

In the AT group, significant reduction in fasting plasma glucose level along with HOMA-IR has been shown. On the other hand, reduction in A1C level by exercise training was significant only in the RT group. Muscle quality as well as muscle mass and strength were significantly increased in both groups. Changes of muscle strength and quality were negatively correlated with the changes of plasma glucose or insulin resistance (for knee flexion,  $r = -0.333$ ,  $p < 0.05$  vs. changes of fasting plasma glucose,  $r = -0.486$ ,  $p < 0.01$  vs HOMA-IR).

#### **Conclusions:**

Both AT and RT increased muscle quality as well as muscle mass or strength in old women with type 2 diabetes. This improved muscle quality was associated with the improvement of insulin resistance.

## METABOLIC ENDOTOXEMIA YOUNG GUERRERO STATE, MEXICO

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### Background:

Studies in mice suggest that lipopolysaccharide (LPS) present in Gram-negative bacteria of the intestine, could be a trigger for low-grade inflammation with impact on weight gain and insulin resistance, however, has not been tested in humans.

### Objective:

Metabolic endotoxemia evaluate obese children and their relationship to elevated lipid levels and decreased Gram negative bacteria.

### Design:

We studied 30 obese subjects and 30 with normal weight, with an age of 18-25 years. Intestinal bacteria were quantified by real time PCR. Endotoxin test was determined with the QCL-1000 Lonza, and biochemical profile was performed under a standard protocol of Spinreact.

### Results:

obese individuals had a BMI of 34.5 (32.9-36.4) kg/m<sup>2</sup>, increased triglyceride concentration (123 [95.5-156.5] mg/dL), total cholesterol (168 [151-194.5] mg/dL), LDL-c (114 [107-149.5] mg/dL), and a slight increase in body temperature (1°C higher than the normal body weight). The reduction of Gram negative bacteria (*E. coli*) was associated with increased serum circulating LPS (1.0 to 1.3 EU/mL). Significantly positive correlation was found between the LPS with BMI ( $r = 0.426$ ,  $p = 0.015$ ), waist circumference ( $r = 0.347$ ,  $p = 0.040$ ) and triglycerides ( $r = 0.415$ ,  $p = 0.001$ ), being higher in obese women. Also found an increase in Gram-positive bacteria in the obese group (*Lactobacillus* and *C. leptum*,  $p < 0.050$ ).

### Conclusion:

The Gram-negative bacteria decreased accompanied by high triglyceride levels and a buildup of circulation central adiposity (waist) may be indicative of a metabolic endotoxemia.

## INSULIN RESISTANCE AND DIABETES MELLITUS ARE FREQUENT COMPLICATIONS IN LONG TERM FOLLOW-UP OF PATIENTS WITH TRAUMATIC BRAIN INJURY

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### Background:

Cognitive impairment and pituitary disease (Pd) are increasingly associated with traumatic brain injury (TBI). We also investigated TBI association with insulin resistance (IR) and diabetes mellitus (DM).

### Methods:

Outpatients were fasting for Siemens PET (ECAT Exact 47) or SPECT (ECAM) brain scans. Indices of cortical metabolism (CMi), perfusion (CPi) and cerebral flow reserve (FRi) used FDG or Tc-99m labeled tracers and perfusion stimulants: omega 3 and/or coconut oils oral, nitroglycerin sl or acetazolamide IV. Test Your Memory (TYM) and Montreal Cognitive Assessment (MoCA) monitored cognition.

### Results:

Among patients without IR or DM before TBI, at (21.6+-14.0) years after TBI 39.4% (52/132) had IR and after (34.3+-15.9) years 29.5% (39/132) had DM. Among TBI patients with IR, 40.4% (21/52) had Pd and 13.5% (7/52) had strokes vs. 48.7% (19/39) Pd, 15.4% (6/39) strokes in TBI patients with DM; and 31.7% (13/41) Pd, 4.9% (2/41) strokes in TBI patients without DM or IR. Among 41 near normal patients FRi was (10.4+-2.3)%. TBI patients with IR had FRi - (0.7 +-6.4)%; with DM - (1.1+-6.5)% and with neither DM nor IR +(2.8+-6.9)%. All stimulant effects were similar, except in 35 repeated studies combined coconut and fish oils increased FRi (3.7+-8.0)%,  $p < 0.02$  vs. other stimulants. TYM and MoCA scores related directly to (FRi) and indirectly to repeated TBI.

### Conclusions:

TBI contributes eventually to a remarkable incidence of IR and DM as well as Pd. Cognition in TBI patients correlates with the extent of endocrinopathy, with stroke incidence and with functional brain imaging abnormalities.

## NAT1 DEFICIENCY IMPAIRS MITOCHONDRIAL FUNCTION AND PROMOTES INSULIN RESISTANCE

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Insulin resistance is a cardinal feature of type 2 diabetes and a potential risk factor for cardiovascular disease. Our recent genome-wide association study (GWAS) has identified N-acetyltransferase 2 (*NAT2*) as a new candidate gene for insulin resistance. *NAT2* (and the mouse homolog *Nat1*) encode a xenobiotic metabolizing enzyme that catalyzes the biotransformation of various drugs and carcinogens but has not previously been linked to insulin resistance. The aim of this study was to evaluate the role of *Nat1* with *in vitro* insulin resistance related phenotypes. Silencing of *Nat1* in differentiated 3T3-L1 adipocytes resulted in increased basal and isoproterenol-stimulated lipolysis and reduced insulin-stimulated glucose uptake. In addition *Nat1* knockdown decreased the expression of adipogenic marker genes and adipocyte differentiation. Furthermore, *Nat1* silencing led to increased production of intracellular reactive oxygen species (ROS) and decreased mitochondrial membrane potential. Collectively, our results suggest that perturbations in *Nat1* affect insulin resistance related endophenotypes and support a causal role for *Nat1* in insulin sensitivity.

### Keywords:

*NAT2*; *Nat1*; 3T3-L1 adipocyte; Insulin resistance; Mitochondrial dysfunction

## PANCREATIC FAT, INDEPENDENT OF SUBCUTANEOUS, VISCERAL AND LIVER FAT, EXPLAINS ELEVATED RISK FOR METABOLIC DISEASE IN AFRICAN AMERICANS BUT NOT HISPANICS

Alderete TL, Toledo-Corral CM, Weigensberg MJ,  
and Goran MI

### Background:

Studies suggest that type 2 diabetes (T2D) risk is related to visceral adipose tissue (VAT) liver fat (LF) and/or pancreatic fat (PF); however, these depots are highly correlated, making it difficult to determine their exact contribution to risk.

### Objective:

To determine which fat depot(s), subcutaneous abdominal adipose tissue (SAT), VAT, LF, and/or PF, is most strongly associated with risk for T2D in minority youth.

### Methods:

216 African Americans (AAs) and Hispanics (BMI percentile 96.2±3.7; age 14.3±2.5 years) were phenotyped for SAT, VAT, LF, and PF by MRI. Clinical fasting glucose (FG), insulin sensitivity (SI), and acute insulin response (AIR) were obtained from FSIVGTT with Minimal Modeling and insulin area under the curve (insAUC) from OGTT. We report standardized  $\beta$ s from regression models including SAT, VAT, LF, and PF (covariates: age, sex, pubertal stage).

### Results:

In AAs and Hispanics, LF was associated with a lower log SI ( $\beta_{AAs}=-0.33$ ,  $\beta_{Hispanics}=-0.29$ ;  $P_{adj}<0.05$ ) and higher log AIR ( $\beta_{AAs}=0.29$ ,  $\beta_{Hispanics}=0.30$ ;  $P_{all}<0.05$ ). In AAs, but not Hispanics, after accounting for SAT, VAT, and LF, those with PF at the 75<sup>th</sup> vs. 25<sup>th</sup> percentile had 2.7% higher predicted FG ( $P_{ethnicity*PF}\leq 0.01$ ) and 16.6% lower predicted AIR ( $P_{ethnicity*PF}=0.07$ ). AAs with PF at the 75<sup>th</sup> vs. 25<sup>th</sup> percentile had 13.2% lower predicted insAUC and Hispanics had 5.1% higher predicted insAUC ( $P_{ethnicity*PF}=0.04$ ).

### Conclusion:

LF was associated with a lower SI and higher AIR in AAs and Hispanics. In AAs, but not Hispanics, PF was related to a higher FG and lower AIR and insAUC. These results suggest that PF may contribute to metabolic dysfunction in AAs.

## SAXAGLIPTIN REDUCES A1C AND IS WELL TOLERATED IN PATIENTS WITH TYPE 2 DIABETES AND HIGH FRAMINGHAM CARDIOVASCULAR RISK OR ALBUMINURIA

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High cardiovascular (CV) risk and albuminuria are common in patients with type 2 diabetes mellitus (T2DM) and make treatment more complex. To assess the efficacy and safety of saxagliptin (SAXA) in patients with T2DM and high CV risk or albuminuria, a post-hoc analysis of data pooled from 5 placebo (PBO)-controlled 24-week studies (2 of SAXA 5 mg/d monotherapy and 1 each of SAXA 5 mg/d add-on to metformin, glyburide, or thiazolidinedione)

stratified by Framingham 10-year CV risk score (<20% vs ≥20%) or albumin/creatinine ratio (ACR) (<30 vs ≥30 mg/g) was performed. Patients (52% women, 68% white) had a mean age of 55 y and mean BMI of 30 kg/m<sup>2</sup>. Week 24 improvements in glycated hemoglobin (A1C), fasting plasma glucose, 2-hour postprandial glucose, and the proportion of patients with A1C<7% were greater with SAXA vs PBO and did not differ across CV risk score or ACR groups. SAXA–PBO differences (95% CI) in adjusted mean changes from baseline in A1C at 24 weeks for CV risk score <20% and ≥20% were –0.54% (–0.67, –0.41) and –0.81% (–0.94, –0.67), respectively, and for ACR <30 and ≥30 mg/g were –0.68% (–0.80, –0.57) and –0.63% (–0.83, –0.44). The proportions of patients with adverse events (AEs, 70%–74%), serious AEs (2%–7%) and symptomatic confirmed hypoglycemia (fingerstick glucose ≤50 mg/dL, 0–1%) were similar across treatment and CV risk and ACR groups. SAXA was effective in patients with T2DM and high Framingham CV risk score or albuminuria and did not increase hypoglycemia risk.

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**Author statement:**

If chosen a poster presentation will be prepared, and, if awarded a high ranking, an oral presentation will be prepared.

**EFFICACY AND SAFETY OF SAXAGLIPTIN IN PATIENTS WITH TYPE 2 DIABETES MELLITUS AND CARDIOVASCULAR DISEASE HISTORY OR CARDIOVASCULAR RISK FACTORS**

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Dipeptidyl peptidase-4 inhibitors raise prandial incretin levels, producing a glucose-dependent increase in insulin and decrease in glucagon secretion to offset insulin resistance in type 2 diabetes (T2DM). Post-hoc analyses of clinical trials of saxagliptin 5 mg/d vs placebo as add-on to metformin (Study 1, NCT00327015), vs glipizide as add-on to metformin (Study 2, NCT00575588), and vs placebo as add-on to insulin±metformin (Study 3, NCT00757588) evaluated glycemic efficacy and safety in T2DM patients with (1) history vs no history of cardiovascular disease (CVD), (2) ≥2 vs 0–1 cardiovascular risk factors, (3) statin use vs no use, and (4) hypertension vs no hypertension. There were no clinically relevant treatment-by-subgroup interactions for change from baseline glycated hemoglobin (HbA1c) with saxagliptin. Adjusted mean (95% CI) treatment differences in HbA1c with vs without CVD history

were –0.38% (–0.91% to 0.15%) vs –0.56% (–0.77% to –0.36%; P=0.53) in Study 1, 0.21% (–0.04% to 0.46%) vs 0.06% (–0.06 to 0.18; P=0.28) in Study 2, and –0.23% (–0.57% to 0.10%) vs –0.48% (–0.68% to –0.27%; P=0.22) in Study 3. Pairwise comparisons by number of risk factors, statin use, and hypertension yielded interaction P-values (across studies) of 0.07–0.84, 0.56–0.98, and 0.34–0.89, respectively. Overall AE rates with saxagliptin (47.1%–67.2%) were lower than or similar to comparator (52.5%–73.0%) across subgroups. Rates of confirmed hypoglycemia were 0–7.8% with saxagliptin and 0–10.1% with comparator. In conclusion, saxagliptin was effective and well tolerated, with low rates of hypoglycemia, irrespective of CVD history or cardiovascular risk factors. Funded by Bristol-Myers Squibb and AstraZeneca.

**Author statement:**

If chosen a poster presentation will be prepared, and, if awarded a high ranking, an oral presentation will be prepared.

**LIPOPROTEIN SUBFRACTION DISTRIBUTIONS IN ADOLESCENTS WITH PCOS SHOW AN ATHEROGENIC PATTERN WHICH IS DISTINCT FROM DISTRIBUTIONS IN ADOLESCENTS WITH TYPE 2 DIABETES**

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**Introduction:**

Polycystic Ovarian Syndrome (PCOS) includes oligomenorrhea and hyperandrogenism, and is associated with insulin resistance (IR) and potentially cardiovascular disease (CVD). IR and CVD are also hallmarks of type 2 diabetes (T2D). We hypothesized that lipoprotein subfraction distributions, markers of CVD risk, would be similarly atherogenic in obese girls with PCOS or T2D compared to obese controls (OC).

**Methods:**

Fasting plasma samples were collected from 52 sedentary obese female adolescents (age 15.5±2 years, 17 PCOS, 20 T2D, and 15 OC). Lipoprotein cholesterol distribution was assessed by fast protein liquid chromatography (FPLC). IR was assessed with a hyperinsulinemic-euglycemic clamp. Group comparisons were performed using difference plots. Significant differences in subfractions are reported when the 95% confidence intervals did not include zero.

**Results:**

Compared with OC, PCOS girls had more cholesterol in small dense LDL subfractions, less in HDL2, and more in HDL3. In contrast, T2D girls had less cholesterol in HDL3, and more distributed as VLDL compared to OC. A direct comparison between PCOS and T2D showed that PCOS had less cholesterol distributed in VLDL, less in large LDL, more in small dense LDL, and more in HDL3. IR was highest in T2D followed by PCOS and OC.

**Conclusion:**

Although both PCOS and T2D girls are characterized by IR and dyslipidemia vs. OC, lipoprotein subfraction cholesterol analysis shows unique profiles in T2D vs. PCOS. Understanding these subtle differences in plasma lipids of adolescents with PCOS and T2D may help to guide treatment aimed at reducing CVD in these populations.

**Funding:**

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**EFFICACY AND SAFETY OF AMG 145, A FULLY HUMAN MONOCLONAL ANTIBODY TO PCSK9: DATA FROM FOUR PHASE 2 STUDIES IN OVER 1200 PATIENTS**

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**Background:**

In recent phase 2 trials, AMG 145, a fully human monoclonal antibody to PCSK9 administered subcutaneously Q2W or Q4W, demonstrated marked reductions in LDL-C, with favorable changes in other lipids.

**Objective:**

To assess the efficacy and safety of AMG 145 from a pooled analysis of four phase 2 trials.

**Methods:**

The 4 trials enrolled 1359 patients. The efficacy analysis included 1252 patients: 951 received AMG 145 and 301, placebo SC. The safety analysis included 1314 patients; 981 received AMG 145, alone or with ezetimibe; 333 received SC placebo, alone or with ezetimibe. Treatment duration was 12 weeks in all trials; primary end point was percentage change in LDL-C by ultracentrifugation (UC) from baseline to week 12. Serious adverse events (SAEs) and other safety data were collected.

**Results:**

Mean (SE) changes in LDL-C from baseline vs placebo at week 12 ranged from -40% (2%) to -59% (2%) across AMG 145 doses, P<0.001 for all dose groups. Favorable changes were observed in apolipoprotein B, lipoprotein(a), triglycerides, HDL-C, and apolipoprotein A1. Higher doses or more frequent administration produced greater efficacy (Table). AEs were more frequent with AMG 145 vs placebo (57% vs 49%). SAEs occurred in 2% vs 1% of AMG 145 vs placebo patients. None were considered treatment related. Muscle-related AEs occurred in 6% vs 4% and CK elevations in 1% vs 1% AMG 145 vs placebo patients.

**Conclusion:**

In this large pooled analysis, AMG 145 dosed either Q2W or Q4W demonstrated significant reductions in LDL-C and favorable changes in other lipids, and was well tolerated over the 12 week treatment period.

*This study was funded by Amgen Inc.*

Table: Week 12 Percentage Change from Baseline		
Parameter	AMG 145 Treatment Parameter Difference vs Placebo,%	
	140 mg Q2W (n=123)	420 mg Q4W (n=213)
LDL-C (by ultracentrifugation)	-59%*	-53%*

Apolipoprotein B	-52%*	-44%*
Lipoprotein(a)	-31%*	-27%*
Triglycerides	-26%*	-16%*
HDL-C	9%*	6%*
Apolipoprotein A1	4%**	4%*
*P<0.001;**P<0.05		

### DECREASED B-CELL FUNCTION IN OVERWEIGHT LATINO CHILDREN WITHIN NORMAL FASTING GLUCOSE PARAMETERS

Monet Jimenez, Tanya Alderete, Michael Goran, Marc Weigensberg, and Claudia Toledo-Corral

#### Objective:

To assess insulin sensitivity (SI), acute insulin response (AIR), and  $\beta$ -cell function (using disposition index, DI) in overweight and obese Latino children with fasting plasma glucose (FPG) below the recommended threshold for pre-diabetes.

#### Methods:

182 healthy overweight Latino children, ages 7-14, with family history of type 2 diabetes were divided into one of three FPG risk groups: low:  $\leq 90$ mg/dL, elevated: 91-99mg/dL, and prediabetic: 100-125mg/dL. SI, AIR, and DI were assessed using FSIVGTT and minimal modeling. Total percent body fat by DEXA and visceral fat by MRI.

#### Results:

AIR and DI decreased significantly across the three FPG groups (AIR,  $p=0.011$ ; DI,  $p=0.023$ ), after controlling for age, pubertal stage, sex, total percent body fat, and visceral fat, while SI was not significantly different ( $p>0.05$ ). The elevated and prediabetic risk groups had significantly lower AIR and DI when compared to the low risk group (all  $p<0.05$ ); however there were no significant differences in AIR or DI between the elevated group and the high risk group (AIR,  $p=0.236$ ; DI,  $p=0.128$ ).

#### Conclusion:

The metabolic profiling of our proposed FPG groups suggests that pancreatic  $\beta$ -cell dysfunction may manifest within current normal FPG parameters in overweight and obese Latino children. In conjunction with other literature in adults and children, research committees should re-evaluate the current threshold of FPG criterion for pre-diabetes.

### OBSTRUCTIVE SLEEP APNEA (OSA) AND SLEEP-DISORDERED BREATHING (SDB) ARE THE PERIPHERAL MANIFESTATIONS OF SYSTEMIC INFLAMMATION

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OSA/SDB are prevalent disorders associated with significant cardiovascular and metabolic complications. Frequent association with metabolic syndrome, obesity, insulin resistance (IR), diabetes mellitus (DM), and hypertension (HTN) suggests that a common denominator is systemic inflammation. We demonstrate that OSA/SDB coexist or are preceded by a systemic, proinflammatory state with metabolic and hormonal dysregulation.

#### Methods:

12 patients (pts), 9 males (M), 3 females (F), underwent CT scans of the neck. OSA was diagnosed by polysomnography (Apnea-Hypopnea Index =  $AHI>5$ ). Accompanying co-morbidities were defined by BMI, % body fat, fasting and postprandial glucose and insulin levels as indicators of IR. Vitamin D3, estradiol (E), and testosterone (T) were obtained to demonstrate nutritional and hormonal imbalances.

#### Results:

CT scan of the neck 12/12 pts (9M, 3F) showed an anterior-posterior pharyngeal diameter  $\leq 7$ mm due to soft tissues hypertrophy.

10/12 had OSA ( $AHI>5$ ) and required CPAP titration.  
2/12 post uvuloplasty showed soft tissue edema, inflammation and airway narrowing.  
12/12 had HTN, DM, obesity or asthma.  
11/12 had BMI  $>25$   
10/12 % body fat  $>30\%$   
10/12 demonstrated evidence of IR  
7/12 had HTN ( $>140/85$ mmHg)  
12/12 had vitamin D deficiency ( $<50$ ng/ml)  
7/9 M had T deficiency ( $<400$ ng/ml) and 2/3 F had E deficiency ( $<30$ pg/ml)  
2/12 did not have sleep studies, reported poor sleep and fatigue.

#### Conclusion:

Metabolic co-morbidities preceded OSA/SDB and contributed to a common underlying process of

inflammation. A systems approach may be a more effective approach to this model of complexity allowing for a more comprehensive, personalized treatment and prevention of OSA/SDB.

**Keywords:**

OSA, inflammation, IR, systems approach

**ACIPIMOX AMELIORATES INSULIN RESISTANCE AND GLUCOSE INTOLERANCE IN A MOUSE MODEL OF OBSTRUCTIVE SLEEP APNEA**

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**Introduction:**

Obstructive sleep apnea (OSA) is associated with insulin resistance, glucose intolerance, and type 2 diabetes. Although causal mechanisms mediating these associations are not well defined, hypoxia-mediated increase in lipolysis in adipose tissue might be involved. Thus, we investigated the effect of acipimox (a lipolysis inhibitor) on glucose tolerance and insulin sensitivity in mice exposed to intermittent hypoxia (IH).

**Methods:**

C57BL/6/J mice were exposed for 14 days to IH or intermittent air (IA). IH was administered by decreasing the FiO<sub>2</sub> from 20.9% to 6.5%, 60 times/hr. For the control animals, IA was administered at an identical rate. Acipimox was administered in the drinking water (0.5 g/mL) during exposure to IH and IA. Following the 14-day exposure, epididymal fat pads were removed and weighted. Primary adipocytes were isolated for assessing lipolysis. Finally, intraperitoneal insulin (0.5 IU/kg) and glucose (1g/kg) tolerance tests were performed.

**Results:**

IH increased fasting glucose by 51% and worsened glucose tolerance and insulin sensitivity by 33% and 102%. IH also increased spontaneous lipolysis by 264%, reduced epididymal fat mass by 15% and adipocyte size by 8% while total body weight remained unchanged. Acipimox averted the IH-induced increase in spontaneous lipolysis and increased epididymal fat mass and adipocytes size by 19% and 10%, respectively. Furthermore, acipimox

completely prevented IH-induced impairments in fasting glycemia, glucose tolerance, and insulin sensitivity.

**Conclusions:**

IN-induced enhanced lipolysis may contribute to insulin resistance and glucose intolerance observed in OSA. Acipimox ameliorated metabolic consequences of IH and may represent a novel treatment option for OSA-induced metabolic disturbance.

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**INCRETIN-BASED THERAPY REVERSES METABOLIC SYNDROME MARKERS IN HIGH-FAT DIET RATS**

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**Introduction:**

In spite of good effects of incretin in diabetic patients, its role in metabolic syndrome (MetS) is largely unknown. Aim of this study to investigate the potential role of exenatide in the cardiometabolic disorders induced by high-fat diet (HFD) in rats and to explore the molecular mechanism driving this activity. Exenatide is a synthetic version of exendin-4, it is a 39 amino acid agonist of the glucagon-like peptide 1 receptor and it is one of the incretin-based therapies.

**Methods:**

Sixty adult male albino rats were used, 40 of them were placed on HFD for 60days for MetS induction. HFD rats were then divided into: HFD vehicle and HFD with exenatide supplementation for 4 weeks. At the end of the experiment, serum was removed from all rats for biochemical study of MetS markers including glucose, insulin, lipid profiles (TC, TG, LDL-C and HDL-C), inflammatory markers (hs-CRP, interleukin-6 and adiponectin) and oxidative stress markers (malondialdehyde and antioxidant enzymes CAT, SOD and GPx). Body weight and insulin resistance (IR) calculated by HOMA were also measured.

**Results:**

HFD induced MetS appeared as obesity, dyslipidemia, IR, significantly higher inflammatory markers and lower adiponectin levels, and lower antioxidant enzymes levels compared to the control rats. Exenatide supplementation to HFD rats abolished several detrimental effects of HFD; it decreased hyperglycemia, hyperinsulinemia, IR, attenuated inflammatory response, oxidative stress and IR, and improved dyslipidemia.

**Conclusion:**

results demonstrate the potential beneficial effects of exenatide on MetS. Thus, incretin based therapy may be an effective therapeutic strategy for MetS treatment.

### ACANTHOSIS NIGRICANS: IMPLICATIONS OF METABOLIC DYSFUNCTION IN POLYCYSTIC OVARY SYNDROME

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**Context:**

The phenotypic heterogeneity in polycystic ovary syndrome (PCOS) encompasses a significant risk for developing type 2 diabetes and increased 'cardiometabolic disease'. Acanthosis nigricans (AN) properly assigns such morbid enclacements of defective homeostasis.

**Objective:**

To further understand the prevalence and interrelations of AN with metabolic dysfunction in PCOS.

**Method:**

Prospective descriptive analysis. A hundred consecutive PCOS patients were diagnosed based on the latest 2003 Rotterdam consensus and the metabolic syndrome (MS) according to the classification stipulated by Grundy et al. (2005). AN was identified by the presence of dark, thick, velvety, pigmented skin, in most cases in the neck. The homeostatic model assessment of insulin resistance (HOMA-IR), glycated hemoglobin, basal insulin and testosterone, total and free, comprise the evaluation. Furthermore, clinical aspects and the cutaneous profile inherent to PCOS were included.

**Results:**

The diagnosis of PCOS consisted, without exception, under full phenotypic expression (hyperandrogenism, chronic anovulation, polycystic ovaries typed). Prevalence of AN (53%), dominant in the neck, over 90% (apart or in combination with other areas), accompanied by hirsutism (72%) and acne (49%), showed significant correlation ( $p < 1\%$ ) with high body mass index (mean value =  $30.63 \pm 9.31$  kg/m<sup>2</sup>), hirsutism ( $p = 0.02$ ), hyperinsulinemia ( $p < 1\%$ ), HOMA-IR (diabetes off)  $\geq 1.18$  ( $p < 1\%$ ), MS ( $p < 1\%$ ); unlike menstrual abnormalities ( $p = 0.11$ ), prevailing amenorrhea (63%), acne ( $p = 0.91$ ), testosterone, total ( $p = 0.19$ ) and free ( $p = 0.07$ ), glycated hemoglobin ( $p = 0.18$ ) and fast glycemia ( $p = 0.15$ ).

**Conclusion:**

Regarding PCOS, considering obesity coexistence, routine inspection of AN, mainly in exposed areas (neck), should be implemented as one of the visible signs of metabolic dysfunction.

### PHARMACOLOGICAL INHIBITION OF RAS PROVIDES MULTIFACET CARDIOPROTECTION IN IN VIVO MODEL OF ISCHEMIA-REPERFUSION INJURY

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**Introduction:**

Angiotensin converting enzyme inhibitors (ACEIs) has been shown to increase survival after myocardial infarction (MI), but the mechanism of action have not been fully characterized at molecular level. Several studies support/oppose the role of angiotensin II type 1 receptor blockers (ARBs) in MI, thus reflecting a paradox. We examined the effects and molecular mechanisms of ACEI ramipril and ARB irbesartan in ischemia-reperfusion (I/R) induced MI in rats.

**Methods:**

Ramipril at 1.25, 2.5 & 5 mg/kg/day and irbesartan at 15, 30 & 60 mg/kg/day were administered orally to rats for 14 days. On 15th day, animals underwent one stage ligation of left anterior descending coronary artery for 45 min and subsequent 60 min reperfusion.

**Results:**

When compared with I/R group, both the treatment groups preserved cardiac functions ( $\pm$ dP/dt, LVEDP, MAP and HR). Morphological studies showed structural derangement and TUNEL assay revealed myocyte apoptosis in ischemic reperfused hearts. Ramipril at all the doses and irbesartan at two lower doses limited the structural loss. The protein profile showed upregulation of  $\alpha$ B-crystallin by ramipril, Hsp27 by irbesartan and  $\beta$ -catenin and cyclin D1 by both drugs.

**Conclusion:**

This study identifies several potential proteins which may help to explain the mechanism of increased survival by ramipril and irbesartan. Hsp27 and  $\alpha$ B-crystallin stabilizes sarcomere that preserved cardiac functions.  $\beta$ -catenin maintained intermyocyte coupling, cytoskeletal stabilization and via upregulating cyclin D1 mediates adaptive hypertrophic response. Further, the differential effect of

these medications on fibroblast apoptosis and proliferation maintains equilibrium between infarct healing and remodeling.

#### **TOLERANCE INDUCTION OF AUTORREACTIVE CD4+ T LYMPHOCYTES RECOGNIZING INSULIN AND GLUTAMIC ACID DECARBOXYLASE FROM TYPE 1 DIABETIC PATIENTS**

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Julio César Ramírez-Reyes<sup>4</sup>, Raquel Faradji<sup>5</sup>,  
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Tolerogenic dendritic cells (tDC) constitute a promising therapy for autoimmune diseases, since they can anergize T lymphocytes recognizing self-antigens in vitro and in vivo. Patients with type 1 diabetes (T1D) have autorreactive T cell clones against pancreatic islet antigens (i.e. insulin, 65 kDa isoform of glutamic acid decarboxylase -GAD65-). We aimed to determine the ability of tDC derived from T1D patients to inactivate their insulin- or GAD65-specific T cells. We isolated CD14+ monocytes and CD4+CD45RA-effector/memory lymphocytes from 12 T1D patients. Immunogenic (cDC) and tDC were generated from monocytes, left unpulsed, or loaded with insulin or GAD65. Then, DC were cultured with autologous memory cells for 5 days (primary culture), and cell viability, proliferation, and IL-2 secretion were determined. These lymphocytes were then re-challenged with unpulsed cDC or cDC loaded with insulin or GAD65 (secondary culture) in order to assess whether the tDC had tolerizing effects on lymphocytes recognizing diabetogenic antigens. In the primary culture, tDC induced lower lymphocyte proliferation (insulin  $p < 0.0004$ , GAD65  $p < 0.001$ ) and interleukin (IL)-2 secretion than cDC in each condition. We noticed that the lymphocytes from a group of patients had high basal proliferation in the absence of antigen (group 1, 5 patients), whereas the lymphocytes from other patients had low basal

proliferation (group 2, 6 patients). The secondary cultures showed that the anergy induction in the group 1 was only effective against insulin ( $p < 0.02$ ), while it was highly robust against insulin ( $p < 0.006$ ) and GAD65 ( $p < 0.002$ ) in the group 2. Importantly, the tolerance induced by tDC against insulin or GAD65 was antigen-specific. These results suggest that tDC therapy against multiple antigens might be useful in a subset of T1D patients.

#### **USE OF PHENTERMINE/TOPIRAMATE EXTENDED-RELEASE (PHEN/TPM ER) IN OBESE/OVERWEIGHT SUBJECTS WITH DYSGLYCEMIA**

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The ADA and AACE recommend weight loss (WL) to improve dysglycemia in obese individuals. PHEN/TPM ER use led to significant WL in obese/overweight adults with  $\geq 2$  weight-related comorbidities in the 56-week, Phase 3 CONQUER study, which randomized subjects to lifestyle modification plus placebo ( $n=994$ ), PHEN 7.5 mg/TPM ER 46 mg (7.5/46;  $n=498$ ), or PHEN 15 mg/TPM ER 92 mg (15/92;  $n=995$ ). This analysis assessed percent WL and change in glycemic parameters in subjects with prediabetes ( $n=1119$ ) and type 2 diabetes mellitus (T2DM;  $n=388$ ) in the CONQUER study. In a separate 56-week, Phase 2 study, obese/overweight subjects with a diagnosis of T2DM controlled with diet and/or oral medications (60% of whom had had T2DM for  $\geq 5$  years and were taking  $\geq 2$  antidiabetic medications) were randomized to placebo ( $n=55$ ) or 15/92 ( $n=75$ ). These 2 studies allowed for a spectrum of subjects with dysglycemia to be evaluated. After 56 weeks of treatment in each study, PHEN/TPM ER induced significant least-squares (LS) mean percent WL vs placebo in all groups ( $P < 0.0001$ , all comparisons; ITT-LOCF). Significant improvements in glycemic parameters were also observed (Table). The most common adverse events among CONQUER subjects with dysglycemia were dry mouth, constipation, and paraesthesia. The incidence of hypoglycemia was low and did not differ from incidence with placebo. No drug interactions were observed between PHEN/TPM ER and metformin or DDP-IVs. WL associated with PHEN/TPM ER, in combination with lifestyle modification, may lead to significant improvements in glycemia compared with placebo, across a range of subjects with dysglycemia.

**Table. LS Mean Change in Weight and Glycemic Parameters Stratified by Glycemic Status (ITT-LOCF).**

			Baseline Mean	LS Mean Change		
				Placebo	PHEN/TPMER 7.5/46	PHEN/TPMER 15/92
CONQUER	Prediabetes (n=1119)	Weight loss	103.9 kg	-2.2%	-8.9%*	-11.0%*
		Fasting glucose, mg/dL	103.9	-1.5	-3.7†	-5.1*
		Fasting insulin, $\mu$ IU/mL	18.2	2.6	-4.0†	-4.5*
		HbA <sub>1c</sub> , %	5.7	0.06	-0.03*	-0.04*
	T2DM (n=388)	Weight loss	100.6 kg	-1.9%	-6.8%*	-8.8%*
		Fasting glucose, mg/dL	133.9	-5.6	-9.7	-11.9
		Fasting insulin, $\mu$ IU/mL	21.6	-4.9	-4.8	-5.4
		HbA <sub>1c</sub> , %	6.8	-0.1	-0.4†	-0.4†
Phase 2	T2DM (n=130)	Weight loss	96.3 kg	-2.7%		-9.4%*
		Fasting glucose, mg/dL	173.5	-27.4		-42.1†
		Fasting insulin, $\mu$ IU/mL	11.9	5.9		2.1†
		HbA <sub>1c</sub> , %	8.7	-1.2		-1.6†

\* $P \leq 0.0001$  vs placebo; † $P < 0.05$  vs placebo

Prediabetes was defined as per ADA guidelines; T2DM was defined as a medical history of T2DM at baseline; ITT-LOCF, intent-to-treat and last observation carried forward

#### EVALUATION OF HOMO AND HETEROZYGOUS FREQUENCIES OF RESISTIN C-420G GENE POLYMORPHISM IN NORTH INDIAN ADULT WOMEN HAVING METABOLIC SYNDROME

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#### Background:

The C-420G polymorphism located in the resistin (RETN) promoter has recently been suggested to play an important role in metabolic consequences. The aim of the present

study was to evaluate the homo and heterozygous frequencies of the RETN polymorphism in north Indian adult women having metabolic syndrome.

#### Methods:

We compared women with metabolic syndrome (MetS) to women without metabolic syndrome (Control; wMetS). Genotype frequencies of the RETN gene were performed using PCR-RFLP method and serum resistin level estimated by sandwich ELISA method.

#### Results:

The proportions of genotypes frequency of Resistin C-420G gene polymorphism in control was in Hardy–Weinberg equilibrium ( $\chi^2=0.43$ ;  $p=0.51$ ). The genotype frequencies of wild type CC genotype were 49.27% vs. 40.52% and homozygous mutant GG type, 7.72% vs. 13.38% whereas allelic frequencies of wild type C allele, 70.77% vs. 63.57% and mutant G, 29.23% vs. 36.43% were observed in wMetS and MetS women. The frequencies of homozygous mutant GG ( $p=0.019$ ) and combined (CG+GG) mutant type ( $p=0.050$ ) differed significantly with wild CC genotype while wild type CC and heterozygous mutant CG ( $p=0.173$ ) did not differ significantly among wMetS and MetS women. However, the frequency of the G allele was

differed significantly with wild type C allele between two groups ( $p=0.014$ ). On the other hand, circulating level of resistin was significantly higher in MetS women than in wMetS women ( $13.96\pm 10.03$  vs.  $9.56\pm 6.26$ ; ng/ml).

**Conclusion:**

The present findings revealed that homozygous GG genotype and G allele of resistin were significantly associated with increased risk/or progression of metabolic syndrome.

**ANGIOGRAPHIC PROFILE OF PATIENTS WITH DIABETES MELLITUS**

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**Background and purpose:**

Diabetes mellitus is a well known cause for accelerating atherosclerosis and complex lesions. Earlier studies have established more incidence of multivessel and diffuse vascular disease in diabetics. This study aimed to investigate the type, pattern and extent of coronary artery involvement in diabetics in comparison with nondiabetics.

**Methods:**

A total of 4230 patients admitted in National Institute of Cardiovascular Diseases (NICVD) ,Dhaka; Al-Helal Specialized Hospital , Mirpur and Uro-Bangla Heart hospital ,Dhaka from August 2003 to June 2013 were angiographically documented for coronary artery disease, 2775 of whom were diabetics and 1455 were nondiabetics according to the WHO diabetes criteria (1999). The patients in these two groups were matched for age, sex, and body mass index (BMI). The 75 g oral glucose tolerance test (OGTT) was performed in all patients, for whom blood glucose, glycosylated hemoglobin (HbA1c), triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) were measured. The clinical features and the data from invasive coronary angiographies were compared between the diabetic and nondiabetic coronary artery disease patients.

**Results:**

Diabetic CAD patients had significantly higher waist to hip ratio (WHR) ( $p=0.014$ ), fasting plasma glucose (FPG), 2h plasma glucose (2hPG), glycosylated hemoglobin (HbA1c)

( $p<0.001$ ). Diabetic patients had one-vessel disease less frequently (26.3% vs 49.1%,  $p<0.001$ ), and three-vessel disease more frequently (37.1% vs 22.8%,  $p=0.009$ ). Diffuse lesions were observed 47.4 % in diabetics and 22.7% in non-diabetics. Left main coronary artery (LMCA) involvement was higher in diabetic group 32.8% versus 22.1 % ( $p<0.001$ ) patients. Bifurcation lesions were higher in diabetics 44.8 % versus 12.2%; calcified lesions were observed in 64.5% in diabetics versus 32.1 % in non diabetics 72% had chronic total occlusion involving single vessel, 23% had 2-vessel involvement and 14% had 3-vessel chronic total occlusion (CTO). The corresponding values in non-diabetics were 41%, 20% and 5% respectively ( $p<0.001$ ).

**Conclusions:**

Diabetics were presented with more severe and diffuse angiographically documented coronary artery disease compared to nondiabetics. Diabetes mellitus results in a higher incidence of chronic total occlusions, and more diffuse coronary lesions. LMCA involvement was also higher in diabetics. Majority of diabetics had 3-vessel disease. Complex lesions (bifurcation) and calcification was significantly higher in diabetics.

**Key words:**

angiogram, diabetes, coronary artery diseases

**RELATIONSHIP BETWEEN BODY MASS INDEX (BMI) AND SEVERITY OF CORONARY ARTERY DISEASE (CAD)**

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**Background:**

Obesity is a major risk factor for diabetes, hypertension and (CAD). Relation between BMI and severity of CAD is matter of controversy. Our aim was to study relation between obesity assessed with BMI and severity of CAD assessed by invasive coronary angiography (ICA).

**Methods:**

415 consecutive patients who underwent Invasive Coronary Angiography (ICA) for different indications included in the study. Coronary stenosis  $> 50$  or more considered significant. Severity of CAD was classified into single, two and three vessels disease. Left main affection was classified as separate category. BMI was calculated and patients classified as Normal (BMI 20–24.9 kg/m<sup>2</sup>), overweight (BMI 25–29.9 kg/m<sup>2</sup>) and obese (BMI  $> 30$  kg/m<sup>2</sup>). Findings of ICA compared with classes of BMI.

**Results:**

The mean BMI was  $31.2 \pm 4.8$  kg / m<sup>2</sup>. Clinical characteristics and ICA results are shown in table 1 and 2 respectively. The mean number of diseased vessels was 1.45 in all BMI classes ( $p=0.73$ ). Multivariate analysis revealed no significant association between BMI and severity of CAD.

**Conclusion:**

BMI was not related to severity of CAD. Larger studies are needed to confirm our results

Table 1: patients' clinical characteristics and results of ICA.

	Normal weight N = 89	Over weight N = 121	Obese N= 205	P value
Age Mean $\pm$ SD	65 $\pm$ 8.3	58 $\pm$ 9.1	55 $\pm$ 10.2	NS
Male	58 (73%)	85 (70.3 %)	144(70.2%)	NS
smokers	28(31.5 %)	37 (30.5%)	81(39.5%)	NS
Hypertension	43 (48.3%)	78 (64.4%)	154 (75.2%)	0.000
DM	15 (16.8%)	25 (20.6%)	62 (30 %)	0.001
Hyperlipidemia	41 (46%)	56 (46.3%)	155 (75.6 %)	0.001
3VD	22 (24.7 %)	25 (20.6 %)	48 (23.4%)	0.75
Left main	4 (4.4%)	5 (4.1%)	11 (5.3%)	0.92

Table 2: Multivariate analysis for risk of 3 vessels and left main disease.

	3 vessels disease			Left main disease		
	Odds ratio	CI 95 %	P value	Odds ratio	CI 95 %	P value
Over weigh	1.23	0.65- 2.2	0.75	0.84	0.25-3.28	0.65
Obese	1.12	0.65-2.0	0.82	1.01	0.34-3.65	0.72
Hypertension	0.94	0.52-1.62	0.001	1.05	0.32-1.45	0.6
DM	3.1	1.9-5.7	0.000	1.03	0.35-2.88	0.83
Dyslipidemia	1.1	0.98-2.86	0.002	0.67	0.74-2.92	0.74

**BONE MARROW-DERIVED MESENCHYMAL STEM CELLS (BM-MSCS, OP9 CELLS) DIFFERENTIATE INTO LIPID-ACCUMULATING FAT CELLS IN ASSOCIATION WITH INSULIN SIGNALING [PROTEIN KINASE G-I (PKG-I), RHOA PHOSPHORYLATION, IRS-1 AND PI3-KINASE]: MODEL OF OBESITY AND TYPE-2 DIABETES**

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**Abstract:**

Insulin resistance is a metabolic disorder characterized by complex interactions between multiple signaling pathways. The exact mechanism is not clearly understood. Accumulation of lipid metabolites has been shown to induce defects in insulin signaling through serine/threonine-kinase cascades. Recent studies show that serine-phosphorylation of IRS (insulin-receptor-substrate) shortens its half-life and decreases association with PI3-kinase. Another important serine/threonine-kinase, i.e. PKG, belonging to the AGC-kinase subfamily (also including PKA and PKC), is expressed in many types of mammalian cells. Our laboratory has shown that two isoforms of PKG-I (PKG-I $\alpha$  and PKG-I $\beta$ ) differ dramatically in their cellular functions. Interestingly, type-1 and type-2 diabetes are associated with dramatic decreases in expression and kinase-activity of PKG-I $\alpha$ , now recognized as key mediator of cardiovascular complications and erectile dysfunction (ED) of diabetes/obesity. In the present study, we investigated ability of BM-MSCs (OP9 cells) to differentiate into lipid-accumulating adipocytes in response to high-insulin and the potential involvement of other insulin-signaling-proteins, PKG-I, RhoA, IRS-1 and PI3-kinase (PI3K). We found that these cells differentiate into lipid-droplet-containing cells within 5 days and that this is associated with dramatic decreases (to only 50%) in protein expression levels of PKG-I (assessed by both Western blot and ultrasensitive quantitative NanoPro 1000), decreases in RhoA serine-188-phosphorylation (site phosphorylated and regulated by PKG-I), and decreases in protein expressions of IRS-1 and PI3K. These changes in PKG-I-catalyzed phosphorylation of RhoA and decreased levels of IRS-1 and PI3K may play a key role in differentiation of BM-MSC into fat cells, a useful model of adipogenesis, obesity and type-2 diabetes.

**A NOVEL FRACTAL ANALYSIS DISTINGUISHES EARLY DEMENTIA FROM MILD COGNITIVE IMPAIRMENT IN INSULIN RESISTANT PATIENTS.**

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**Background:**

Mild cognitive impairment (MCI) and dementia are of increasing importance in insulin resistant (IR) patients.

**Methods:**

Basal and perfusion-stimulated brain scans used Siemens e.cam SPECT or ECAT Exact 47 PET (MiEScintaron) for metabolic, perfusion and flow reserve indices (CMi, CPI, FRi). Modified fractal analysis used average activity, A within isocontours, I = percentages of maximal activity, M. The positive slope, S of ln(A) vs. ln(I) graphs is analogous to the negative slope, D, the fractal dimension, using ln(V), with V = voxels within each isocontour instead of ln(A).

**Results:**

Among 41 near normal patients, M = 2.50±0.17 times average white matter activity and average CMi (59.5±4.6)% = CMa, which helped normalizing A to AN = (A/M) raised to power (CMa/CMi). Ln(AN) vs. ln(I) graphs intercept zero with slope, SN, whose values near the intercept agreed within 10% for PET or SPECT in individual patients. Patients age (60.2±17.9) years, near normal (n = 11), or with IR and MCI (n = 15) or early dementia (n = 7), respectively had CMi (71.8±4.2)%, (60.2±9.7)%, (50.0±5.7)%; FRi (3.90±3.64)%, (0.43±6.5)%, (-0.19±3.74)% and average SN (of basal and perfusion-stimulated values): 0.347±0.053; 0.586±0.086; 0.930±0.155. Analysis of FRi and/or ratios of CPI/CMi may also be effective; however, SN values are more precise, and distinguish MCI from early dementia (p < 0.001) even better than D (p < 0.01) or FRi (p = 0.8).

**Conclusions:**

The modified fractal slope, SN, is the best single parameter we have found to distinguish early dementia from MCI in IR patients.

**INNOVATIVE NANO-PROTEOMIC TECHNIQUE (NANOPRO1000), BASED ON ULTRASENSITIVE CAPILLARY ELECTROPHORESIS (CE), FOR PRECISE QUANTIFICATION OF PROTEIN EXPRESSION AND PHOSPHORYLATION LEVELS OF PRO-SURVIVAL PROTEINS (E.G. PKG-I AND SURVIVIN) IN HUMAN PANCREATIC ISLETS**

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Previously, we showed that PKG-I stimulates cell-proliferation/DNA-synthesis and migration/invasion and protects against apoptotic cell-death in lung-cancer and ovarian-cancer cells. Whereas in some normal cells (e.g. vascular smooth muscle cells) PKG-I expression is easy to detect using conventional Western blots, cancer cells have dramatically lower expression levels, likely due to redistribution of PKG-I from cytosol (main subcellular locale in normal cells) to the membrane, where it interacts with other pro-oncogenic/pro-survival kinases, such as c-Src. PKG-I is also found at perinuclear sites, where it can interact with Survivin, another pro-survival protein. Both PKG-I and Survivin are relatively low-abundance in human islets and are difficult to accurately quantify by conventional Western blotting. To remedy this problem, we are using a novel instrument called NanoPro1000 (ProteinSimple), state-of-the-art instrument based on advanced "nano-proteomics" utilizing ultrasensitive capillary-electrophoresis (CE)-based technologies. NanoPro1000 quantifies protein expression/phosphorylation levels with sensitivities >500-times that of conventional Western blots, thus allowing, for the first time, accurate measurements of lower-abundance proteins, such as PKG-I, Survivin and other pro-survival proteins. Extremely small samples (<100 cells) are required. We found that, using NanoPro1000 technology, human pancreatic islets express PKG-I. This advanced "nano-proteomics" allows for identification of many novel proteins and phosphorylations, which will be used for developing new therapies (based on accurate molecular targeting) for treating diabetes. We are confirming colocalization of PKG-I and Survivin in human islets and, further, using NanoPro1000, determining expression levels and phosphorylation states of many signaling/pro-survival proteins in different cell-types constituting human islets (alpha, beta, delta, gamma, epsilon and pancreatic endothelial cells).