

Abstracts

4th International Symposium on Diabetes Mellitus and its Complications: From Molecular Biology to Clinical Medicine Preston, UK

Na⁺/Ca²⁺ exchange current (I_{Na/Ca}) and sarcoplasmic reticulum (SR) Ca²⁺ release in catecholamine-induced Cardiac hypertrophy

Alzbeta Chorvatova, George Hart and Munir Hussain
Department of Medicine, University Clinical Departments,
University of Liverpool, Liverpool, L69 3GA.

We investigated the effects of cardiac hypertrophy, produced by catecholamine administration, on I_{Na/Ca} and SR Ca²⁺ release in isolated rat left ventricular myocytes. Steady state I_{Na/Ca} density, measured using descending (+80 to -110 mV) voltage ramps in the whole cell configuration, was increased in hypertrophied myocytes (P<0.05). Ca²⁺ release from the SR was also increased, whereas resting [Ca²⁺]_i and the rate of decline of [Ca²⁺]_i to control levels were unchanged. SR Ca²⁺ content, estimated by using 10.0 mmol/L caffeine was also significantly increased in hypertrophied myocytes, but only when myocytes were held and stimulated from their normal resting potential (-80 mV) but not from -40 mV. However, the rate of decline of caffeine-induced Ca²⁺ transients or I_{Na/Ca} was not significantly different between control and hypertrophied myocytes. Ca²⁺-dependence of I_{Na/Ca}, examined by comparing the slope of the descending phase of the hysteresis plots of I_{Na/Ca} vs. [Ca²⁺]_i, was also similar in the two groups of cells. The observation that increased SR function occurred only when myocytes were stimulated from -80 mV suggests that Na⁺ influx may play a role in altering Ca²⁺ homeostasis in hypertrophied cardiac muscle, possibly through increased reverse Na⁺/Ca²⁺ exchange.

Effect of streptozotocin on insulin and glucagon secretion from the isolated rat pancreas.

Ernest Adeghate and Abdulsamad Ponery
Department of Anatomy, Faculty of Medicine and Health Sciences, United Arab Emirates University, P.O. Box 17666, Al Ain, United Arab Emirates.

Streptozotocin (STZ) is one of the most commonly used agents in studies on experimental diabetes. It was the aim of this study to examine the effect of STZ on insulin and glucagon secretion from the isolated rat pancreas. Pancreatic tissue fragments were incubated with different concentrations of STZ for 1 h and the supernatant radioimmunoassayed for insulin and glucagon. Stimulation of the isolated pancreas with STZ (10⁻⁸-10⁻⁴ M) resulted in a dose-dependent increase in insulin secretion. Insulin secreted into supernatant (mean ± standard deviation) was 20.31 ± 2.05, 37.28 ± 7.99 and 41.94 ± 11.42 μIU L⁻¹ when

pancreatic tissue fragments were incubated with 10⁻⁸ M, 10⁻⁶ M and 10⁻⁴ M of STZ respectively. The level of insulin secretion obtained with STZ stimulation at 10⁻⁶ and 10⁻⁴ M were significantly (P < 0.02) higher than that of basal (19.83 ± 5.10 μIU L⁻¹). STZ elicited a dose-dependent increase in glucagons secretion from the pancreas. Glucagon secretion expressed as mean ± standard deviation was 184.75 ± 44.44, 205.83 ± 44.30 and 248.01 ± 98.20 pg mol⁻¹, after stimulation with 10⁻⁸ M, 10⁻⁶ M and 10⁻⁴ M of STZ, respectively. Glucagon secretion at 10⁻⁴ M of STZ was almost a two-fold of that obtained in the basal (156.27 ± 9.52 pg mol⁻¹). In summary, STZ can elicit significant dose-dependent increases in insulin and glucagons secretion from the isolated pancreas. STZ can thus be regarded as a secretagogue of pancreatic hormones.

An update on the aetiology and epidemiology of diabetes mellitus

Ernest Adeghate¹, Peter Schattner² and Earl Dunn³
¹Department of Anatomy, Faculty of Medicine and Health Sciences, United Arab Emirates University, Al Ain, United Arab Emirates and ²Department of Community Medicine and General Practice, Monash University, Australia, and ³Department of Family Medicine, Faculty of Medicine and Health Sciences, United Arab Emirates University, Al Ain, United Arab Emirates.

Diabetes mellitus is one of the most common endocrine disorders affecting almost 6 percent of the world's population. The number of diabetic patients will reach 300 million in 2025 (International Diabetes Federation, 2001). More than 97 % of these patients will have type II diabetes. The projected increase in the number of diabetic patients will strain the capabilities of health care providers the world over. Thus it is of paramount importance to revisit the causes and epidemiology of diabetes mellitus. Diabetes mellitus is caused by both environmental and genetic factors. The environmental factors that may lead to the development of diabetes mellitus include physical inactivity, drugs and toxic agents, obesity, viral infection and location. While Type I diabetes is not a genetically predestined disease, an increased susceptibility can be inherited. Genetic susceptibility plays a crucial role in the aetiology and manifestation of Type II diabetes with concordance in monozygotic twins approaching 100%. Genetic factors may have to be modified by environmental factors for diabetes mellitus to become overt. An individual with a susceptible gene may become diabetic if environmental factors modify the expression of these genes. Since there is an increase in the trend at which diabetes

prevail, it is evident that environmental factors are playing a more increasing role in the cause of diabetes mellitus.

The effect of diabetes mellitus on the morphology of the heart

Adeghate E

Department of Anatomy, Faculty of Medicine and Health Sciences, United Arab Emirates University, P.O. Box 17666, Al Ain, United Arab Emirates

Cardiovascular complication is one of the most common causes of morbidity and mortality in diabetic patients. The mechanisms by which these morphological changes occur are poorly understood. Diabetes mellitus induces abnormal changes in the structure of different components of the heart. These diabetes-induced morphological changes include swelling of mitochondria leading to degeneration in the intra-mitochondrial area. Severe diabetes mellitus is also associated with disarray of the sarcomere and invagination of the nuclear membrane of cardiomyocytes. Some reports show a decrease in the volume fraction of myofibrils, sarcoplasmic reticulum, T-tubules and cardiomyocyte diameter. In diabetes mellitus, the cytoplasm of the cardiomyocytes is laden with vacuoles and lipid droplets decreasing the effective area of the heart for contractility. Diabetes mellitus also affects the cellular components of the cardiac interstitium. The quantity of collagen III, IV and VI is increased in diabetes. In addition many reports indicate that the basement membrane of the endothelium and cardiomyocytes are thickened. The pathological changes observed in cardiomyocytes and in the interstitial areas of the heart may be a result of the accumulation of advanced glycation end products in the wall of blood vessels and in basement membranes. A number of agents including antioxidants and anti-diabetic drugs such as troglitazone have been shown to reduce and or reverse these diabetes-induced morphological changes.

Alterations in Atrial Natriuretic Peptide (ANP) and its Receptors in Streptozotocin induced Diabetic Rat Kidneys

Abdu Adem¹, Ernest Adeghate², Irwin Chandranath¹, Sheela Benedict³, and Enyioma Obineche³ Departments of Pharmacology¹, Anatomy², and Internal Medicine³, FMHS, UAE University, Al Ain, UAE.

The effect of diabetes mellitus on ANP and ANP receptors (ANP-R) of streptozotocin (STZ) induced diabetic rat kidneys was investigated in this study. The levels of ANP receptors in diabetic and control kidneys were evaluated by quantitative receptor autoradiography. Moreover, the distribution of ANP in kidney and heart tissues was studied by immunohistochemistry. In addition, heart and plasma ANP concentration was evaluated in diabetic and control rats using radioimmunoassay. Bodyweight loss was used as an index of diabetes mellitus in the STZ rats. There was significant loss in the bodyweight of the diabetic rats compared to controls. The efficacy of STZ. administration

was confirmed by rising blood glucose levels which were significantly higher in diabetic rats compared with controls. The distribution and levels of ANP-R in diabetic rat kidneys and age-matched controls was investigated using quantitative receptor autoradiography. Our results demonstrate significant decrease in ANP-R in diabetic kidneys compared to controls. The significant decrease was found in the juxtglomerular medulla, inner medulla and the papillae. Intense immunostaining for ANP was only observed in the diabetic heart compared to controls. Heart and plasma ANP concentrations were significantly greater in the diabetic rats compared to controls. The increase in heart ANP concentration could be as a result of plasma volume expansion present in STZ diabetic rats. This increase in heart ANP contributes to the high concentration of ANP observed in the plasma. Such increase in plasma ANP concentrations may result in the downregulation of kidney ANP receptors seen in the diabetic kidney. The decrease in ANP receptors observed in the diabetic rat kidneys might have pathological consequences resulting in renal resistance to ANP in diabetes.

Differential action of hypoglycaemic trace elements on gluconeogenesis in kidney-cortex tubules isolated from control and diabetic rabbits.

Jadwiga Bryla, Anna Kiersztan, Katarzyna Winiarska, Marta Przedlacka, Michal Wegrzynowicz, Tomasz Fraczyk.

Department of Metabolic Regulation, Institute of Biochemistry, Warsaw University, Poland.

Derivatives of vanadium, selenium, tungsten and molybdenum exhibit insulin-mimetic properties when given orally to either diabetic patients or diabetic animals. The action of these trace elements on gluconeogenesis was studied in kidney-cortex tubules isolated from control and alloxan-diabetic rabbits, which exhibit intracellular localization of gluconeogenic enzymes similar to human. Gluconeogenic metabolites and oxidized glutathione (GSSG) were determined either spectrophotometrically or fluorimetrically with the use of specific enzymes, while reduced glutathione (GSH) was assayed by HPLC. The mitochondrial membrane potential ($\Delta\phi$) and the cellular reactive oxygen species (ROS) were measured fluorimetrically with JC-1 and 2'-7'-dichlorofluorescein diacetate, respectively. Rates of gluconeogenesis in renal tubules of diabetic rabbits were increased despite diminished $\Delta\phi$ and the cellular and mitochondrial GSH/GSSG ratios. Vanadyl acetylacetonate (VAc), at 100 μ M concentration, resulted in a marked inhibition of renal gluconeogenesis (by about 60-80%), accompanied by increased ROS production and decreased $\Delta\phi$, the cellular and mitochondrial GSH/GSSG ratios and glucose-6-phosphatase activity. Less pronounced changes in renal glucose synthesis, $\Delta\phi$ and the ROS generation were observed in the presence of tungstate and molybdate, while selenate had no influence. Administration of 2 mM N-acetylcysteine (NAC) partially attenuated vanadium-induced alterations in glucose synthesis, ROS production, $\Delta\phi$ and

cellular GSH/GSSG ratios. In view of (i) a decline in $\Delta\phi$ and cellular GSH/GSSG ratios, probably due to increased ROS generation, accompanying inhibitory actions of V, W and Mo on renal gluconeogenesis and (ii) a partial reversal of the metal-induced changes by NAc, a potential therapeutic application of V-, W- and Mo-compounds needs a careful evaluation.

Epidemiology of Type 2 Diabetes in Indians

Sangram Jadhav
Medical Director, Dr. DY Patil Medical College, Sec 7,
Nerul, Navi, Mumbai, India

Prevalence of diabetes is increasing globally. India has the maximum increase during the last few years. Type 2 diabetes mellitus is the commonest form of diabetes. The prevalence of type 2 diabetes mellitus is 2.4% in rural population and 11.6% in urban population. Prevalence of impaired glucose tolerance is also high in the urban population. Subjects under 40 years of age have a higher prevalence of impaired glucose tolerance than diabetes. The important risk factors for high prevalence of diabetes include: High familial aggregation, obesity especially central one, insulin resistance and lifestyle changes due to rapid urbanisation. Diabetes Research Centre, carried out many studies regarding the risk factors involved in causing type 2 diabetes mellitus. The results of these studies are mentioned here in this article to have a glimpse of overall risk factors involved in causation of diabetes mellitus.

The Prevalence, Type and Severity of Cardiovascular Diseases in Diabetic and Non-Diabetic Patients.

Fallows GD and Singh, J
Department of Biological Sciences, University of Central Lancashire, Preston,
UK and The Department of Cardiology, Victoria Hospital, Blackpool, UK.

Introduction: Diabetes mellitus is a major global health problem and it affects more than 180 million people worldwide. Long-standing diabetic patients are susceptible to microvascular and macrovascular disease. Abundant evidence shows that patients with either type 1 or type 2 diabetes are at higher risk for several cardiovascular disorders: coronary heart disease, stroke, peripheral arterial disease, cardiomyopathy, and congestive heart failure, cardiovascular complications are recognised as the leading causes of diabetes-related morbidity and mortality. People with diabetes have a two to eight fold excess in cardiovascular mortality than people without diabetes.
Aims and Methods : This study compared angiographically-determined cardiovascular disease in 79 patients with Diabetes Mellitus and an equal number of matched controls without diabetes under the age of 55 years.

Seventy-nine diabetic patients coming to coronary angiography during a twelve-month period were reviewed retrospectively along with 79 control patients matched for age (± 3 years), sex, ethnic origin and risk factors

(hyperlipidemia, body mass index and smoking history). The angiographic features of a consecutive series of 62 European and 17 Asian patients and their matched-paired controls were assessed; all study subjects had undergone elective coronary angiography and ventriculography. Angiographic findings were graded to describe severity and extent of coronary atherosclerosis. Left ventricular systolic function was assessed by ejection fraction. **Results:** The diabetic group had a significantly higher arterial systolic pressure than the non-diabetic group ($p < 0.0081$) and they were clinically obese with a body mass index of > 30). Detailed analysis of the angiograms showed that prevalence and severity of coronary artery disease in diabetic patients was greater, the mean 'severity score' was 11.66 for the diabetic group against 8.49 for the non-diabetic group ($p < 0.037$), multivessel disease was more common in diabetic patients than in the controls, with 3-vessel disease being the most common. 38 of 79 diabetic patients had 3-vessel disease compared to 29 of 79 controls. Diabetic patients were also more likely to have more segments diseased in one vessel. Systolic function was reduced in the diabetic group, with a significantly lower ($P < 0.035$) mean ejection fraction. **Conclusion :** The present study supports the evidence that diabetic patients have more extensive coronary artery disease than non-diabetic patients and a poorer prognosis, and that the coronary arteries of the Asian patients were affected more adversely than those of the European group irrespective of the diabetic state.

The English National Risk Reduction Programme for Preservation of Sight in Diabetes

Warren Gillibrand¹, Deborah Broadbent² Simon Harding³ & Jiten Vora⁴.

1. Member, Policy Advisory Group, National Screening Committee, Department of Health, Lecturer: Department of Nursing, University of Central Lancashire, Preston, PR1 2HE, Tel: +44 01772 893775, Fax: +44 01772 892998, E-mail: wpgillibrand@uclan.ac.uk, 2. Member, Policy Advisory Group, National Screening Committee, Department of Health, Director: The Liverpool Diabetes Eye Centre, Royal Liverpool University Hospital, Rowan House, Prescot Street, Liverpool L7 8XP., 3. Member, Policy Advisory Group, National Screening Committee, Department of Health, Consultant Ophthalmologist, St. Pauls Eye Unit, Royal Liverpool University Hospital, Prescot Street, Liverpool L7 8XP., 4. Consultant Endocrinologist, Department of Diabetes & Endocrinology, Royal Liverpool University Hospital, Prescot Street, Liverpool L7 8XP.

Introduction: The National Service Framework for Diabetes has set the first target that all people with diabetes in England will be screened annually for the detection of sight threatening eye disease (STED) by 2007. There is consistent evidence that screening for diabetic retinopathy is effective and treatment modalities have been established which could prevent partial sight and blindness registration in 80-90 % of cases. **Methods:** The Liverpool Diabetic Eye Study uses a mobile screening system utilising 35 mm, (converting to digital) 3-field fundus photography in all

people with diabetes not attending an ophthalmologist. In 1995/1996 we reported detection of STED in 7.4% of cases compared to a rate of 3.7% in 2001/2002, with a sensitivity of 93% and specificity of 94%. The Policy Advisory Group has been formulated with the main aim to establish a cost effective national STED screening programme. There is currently inconsistency in service provision for diabetic retinopathy screening in the UK, ranging from organised services employing cost effective technology to ad hoc services with no central organisation. Two models of service delivery are recommended which adhere to the National Screening Committee's requirements for a screening programme: diabetic retinopathy screening using digital mydriatic retinal photography in both community and secondary care settings with image reading by accredited individuals; diabetic retinopathy screening using indirect slit lamp mydriatic retinal biomicroscopy performed by trained personnel in both community and secondary care settings. **Discussion:** Increased funding for resources is required in some health authority regions in order to establish a screening programme. It is hoped that all people with diabetes will be screened for STED in order to reduce the burden of diabetic retinopathy in society.

Effects of volatile anaesthetics on contraction in ventricular myocytes from streptozotocin-induced diabetic rat

Graham MD, Qureshi MA, Harrison SM, Howarth FC

Various clinically used volatile general anaesthetics (e.g. sevoflurane, halothane, isoflurane & desflurane) have been shown to have significant inotropic effects on normal ventricular muscle (1) however, little is known about their effects in ventricular tissue from diabetic animals. Streptozotocin (STZ)-induced diabetes is known to induce changes in the amplitude and time course of shortening (2,3) and one report suggests that the inotropic effects of anaesthetics are ameliorated in papillary muscles from diabetic animals (4). The aim of these studies was to investigate this further in electrically stimulated (1 Hz) ventricular myocytes. Cells were superfused with either normal Tyrode (NT) solution or NT containing anaesthetic (1 mmol/l) for a period of 2 min (at 30-31 °C). Myocytes from STZ rats were shown to have a significantly longer time to peak ($P > 0.001$, $n = 50$) and shortening tended to be greater but this was not significant ($P = 0.11$, $n = 50$). Halothane, isoflurane, desflurane and sevoflurane significantly ($P < 0.05$) reduced contraction of control cells by $72.5 \pm 3.2\%$, $46.5 \pm 9.7\%$, $28.9 \pm 4.3\%$ and $22.8 \pm 5.6\%$ respectively, ($n > 12$ per group) but their negative inotropic effect was found to be no different in cells from STZ-treated rats ($73.0 \pm 4.8\%$, $40.7 \pm 4.7\%$, $25.0 \pm 5.2\%$ and $19.8 \pm 5.2\%$, respectively) in contrast with previous data (4). Therefore, we conclude that the inotropic effects of volatile anaesthetics were not altered by STZ-treatment.

1. Davies LA *et al*, (1999), *Brit J Anaes*, 82(5): 723-730
2. Choi KM *et al*, (2002), *Am J Physiol*, 283:H1398-H1408
3. Howarth FC *et al*, (2002), *Pflugers Arch*, 444:446-451
4. Hattori *et al*, (1987), *Anaes Analg*, 66: 23-28

Effects of pressure overload-induced hypertrophy on TTX-sensitive inward currents in guinea-pig left ventricle

Alzbeta Chorvatova, Richard Snowdon George Hart and Munir Hussain

Department of Medicine, University Clinical Departments, University of Liverpool, Liverpool, L69 3GA

We investigated the effects of pressure-overload hypertrophy on inward sodium (I_{Na}) and calcium currents (I_{Ca}) in single left ventricular myocytes to determine whether changes in these current systems could account for the observed prolongation of the action potential. Hypertrophy was induced by pressure-overload caused by banding of the abdominal aorta. Whole cell patch clamp experiments were used to measure tetrodotoxin (TTX)-sensitive inward currents. The main findings were that I_{Ca} density was unchanged whereas I_{Na} density after stepping from -80 to -30 mV was decreased by 30% (-9.0 ± 1.16 pA/pF in control and -6.31 ± 0.67 pA/pF in hypertrophy, $P < 0.05$, $n = 6$). Steady-state activation/inactivation variables of I_{Na} , determined using double-pulse protocols, were similar in control and hypertrophied myocytes, whereas the time course of fast inactivation of I_{Na} was slowed ($P < 0.05$) in hypertrophied myocytes. In addition, action potential clamp experiments were carried out in the absence and presence of TTX under conditions where only Ca^{2+} was likely to enter the cell via TTX-sensitive channels. We show for the first time that a TTX-sensitive inward current was present during the plateau phase of the action potential in hypertrophied but not control myocytes. The observed decrease in I_{Na} density is likely to abbreviate rather than prolong the action potential. Delayed fast inactivation of Na^+ channels was not sustained throughout the voltage pulse and may therefore merely counteract the effect of decreased I_{Na} density so that net Na^+ influx remains unaltered. Changes in the fast I_{Na} do not therefore appear to contribute to lengthening of the action potential in this model of hypertrophy. However, the presence a TTX-sensitive current during the plateau could potentially contribute to prolongation of the action potential in hypertrophied cardiac muscle

Enhanced cardiac protein phosphatase activity in type I diabetes

Thomas Netticadan

Institute of Cardiovascular Sciences, St. Boniface Hospital Research Centre and University of Manitoba, Canada

Depressed cardiac function has been reported to occur in type I diabetes, however the underlying mechanisms are not clearly established. Protein phosphorylation and dephosphorylation play important roles in regulating cardiac function. Because protein phosphatases mediate protein dephosphorylation, we hypothesized that cardiac dysfunction induced by diabetes will in part be associated with alterations in the activities of these enzymes. Accordingly, rats were made diabetic by administering a single intravenous injection of streptozotocin (65 mg/kg

body wt) and cardiac function and protein phosphatase activities examined after 1, 2, 3, 4 and 8 weeks. One group of 4-week diabetic animals received subcutaneous injection of insulin (3U/day) for a further period of 4 weeks. Cardiac dysfunction was apparent early on (after 2 weeks of induction of diabetes) and deteriorated with time. A significant increase in protein phosphatase activity appeared very early (after one week) and persisted until 8 weeks. Increased protein phosphatase activity was consistent with a corresponding increase in the protein contents of protein phosphatase 1 and protein phosphatase 2A. Treatment with insulin partly reversed the abnormalities observed in diabetic animals. The results of this study suggest that increased protein phosphatase activities and therefore enhanced protein dephosphorylation may play a role in diabetes induced cardiac dysfunction.

Fructose transport and metabolism in adipose tissue of Zucker rats: diminished GLUT5 activity during obesity and insulin resistance

Gary J. Litherland, Eric Hajduch, Gwyn W. Gould* & Harinder S. Hundal

Division of Molecular Physiology, School of Life Sciences, MSI/WTB Complex, The University of Dundee, Dundee DD1 5EH U.K. and *Division of Biochemistry and Molecular Biology, University of Glasgow, Glasgow, U.K.

Fructose is a major dietary sugar which is elevated in the serum of diabetic humans, and is associated with metabolic syndromes important in the pathogenesis of diabetic complications. The facilitative fructose transporter, GLUT5, is expressed in insulin sensitive tissues of humans and rodents, where it mediates the uptake of substantial quantities of dietary fructose, although little is known about its regulation. Here, we present evidence that GLUT5 expression and activity are compromised severely during obesity and insulin resistance in Zucker (*fa/fa*) rat adipocytes. Adipocytes from young (*fa/fa*), highly insulin-responsive Zucker rats present considerably more surface GLUT5 than those from their lean counterparts (1.8-fold per microgram membrane protein), and exhibit higher fructose transport (4-fold) and metabolism (3-fold) rates, largely directed toward lactate production. As rats age and become more obese and insulin-resistant, adipocyte GLUT5 density (12-fold) and fructose transport (10-fold) and utilisation rates (3-fold) fall markedly. The GLUT5 loss is more dramatic in adipocytes from obese animals, which develop a more marked insulin resistance than lean counterparts. The decline of GLUT5 density in adipocytes from older, obese animals does not appear to be a generalised effect, being neither shared by the Na⁺/K⁺ ATPase, a plasma membrane marker, nor observed in kidney. Our findings suggest that GLUT5 expression and thus fructose utilisation rates in adipocytes are dependent upon cellular insulin sensitivity, inferring a significant role for GLUT5 in the elevated circulating fructose observed during diabetes, and the likely pathological consequences.

The effects of Type 1 diabetes mellitus on the morphology, cytosolic calcium ([Ca²⁺]_i) signals and fatty acid lipid profiles in the isolated rat parotid gland.

S. Mahay^(a), J. Singh^(a), E. Adeghate^(b), M.Z. Lindley^(a) and C.E. Rolph^(a).

^(a)Department of Biological Sciences, University of Central Lancashire, Preston and ^(b)Department of Human Anatomy, F.M.H.S, United Arab Emirates University, P.O. Box 17666, Al-Ain, UAE.

Diabetes mellitus (DM) is associated with numerous conditions including hypo-secretion of digestive enzymes. This study investigates the morphology, [Ca²⁺]_i signals and fatty acid lipid profiles in isolated parotid glands of diabetic and age-matched control rats in order to understand the cellular mechanism of hypo-secretion. The techniques employed included light microscopy, fluorimetric and gas chromatography analysis (GC), respectively. DM was induced in adult male Wistar rats by a single (IP) injection of streptozotocin (STZ) (60 mg kg/ body weight). Control animals were injected with a similar volume of citrate buffer. The animals were tested for DM 4 days after STZ injection and 2 months later when they were humanely killed for the experiment. The morphological results showed diabetic parotid glands to be extensively infiltrated with lipid droplets of various magnitudes, whereas glands from control animals, display normal structure with the absence of lipid droplets. DM produced no significant change in either basal or Ach-evoked initial peak [Ca²⁺]_i signals when compared to age-matched control cells. In contrast, DM induced a significant (P<0.01) reduction in the plateau phase of the [Ca²⁺]_i signal compared to control cells. Levels of fatty acids (16:0, 16:1 and 18:1) in diabetic parotid glands were significantly (P<0.01) reduced compared to control glands. The results indicate DM can elicit changes in the morphology, [Ca²⁺]_i signals and in fatty acid lipid profiles of the isolated rat parotid gland, compared to glands from healthy age-matched control rats.

The effect of Vitamin E on metabolic parameters of Experimental Diabetes Mellitus

¹Al Shamsi MS, ²Amin A and ³Adeghate E

Department of Graduate Studies, Department of Biology, Faculty of Science and Department of Anatomy, Faculty of Medicine, United Arab Emirates University, Al Ain, UAE.

The role of vitamin E on Diabetes Mellitus is unknown. The purpose of this study was to examine the effect of oral administration of vitamin E on some of the metabolic effects of experimental diabetic rats. Diabetes was induced by intraperitoneal injection of STZ (60 mg/kg body weight at 12 weeks of age). Vitamin E (0.2, 0.4, 0.8 mg/Kg body weight) was administered orally for a period of three weeks to normal and diabetic Wistar rats. In some experiments Vitamin E was given either before or after the induction of diabetes mellitus. Oral glucose tolerance test (OGTT) were performed on fasted normal, diabetic and vitamin E-treated rats at the end of the experimental period. Blood sugar level and weight were also recorded on a weekly basis for each

rat in different groups. Vitamin E significantly ($p < 0.01$) reduced blood sugar level and improved weight gain in experimental diabetes mellitus at all doses when compared to untreated rats. This beneficial effect of vitamin E on the hyperglycaemia of diabetic rats was dose-dependent. Moreover, vitamin E also improved OGTT in diabetic rats compared to untreated diabetic rats. In conclusion, Vitamin E may play a role in insulin metabolism and thus be a useful adjuvant therapy in type I diabetes mellitus.

The time course of changes in amine concentrations in the rat tail artery following induction of diabetes with streptozotocin.

John Morrison, S Dhanasekaran and Rajan Sheen, Departments of Physiology and Pharmacology, UAE University, Al Ain, UAE

Introduction: We have examined the changes in concentrations of noradrenaline, dopamine and serotonin in the proximal, middle and distal segments of the rat tail artery following the induction of diabetes by injection of STZ (60 mgs/kg ip at 10 weeks of age); the present abstract is concerned with the time course of the changes from 17.5 weeks of age to 52 weeks of age. Tissues were taken under pentobarbitone anaesthesia, weighed and treated with 10% sodium metabisulphite. Amines were extracted and analysed using HPLC. **Results:** The control blood glucose concentrations were in the range 57 to 68 mgs/dl and the diabetic levels were 227 to 380 mgs/dl. In the control animals there was a significant increase in noradrenaline, dopamine and serotonin concentrations with age in the proximal portion of the tail artery, whereas this was not seen in the middle (except in the case of serotonin) and distal segments. Between 20 and 30 weeks of age, noradrenaline was the most abundant amine, followed by serotonin, dopamine and adrenaline. STZ diabetes resulted in major increases in the concentrations of noradrenaline, dopamine and serotonin (4-11fold) that generally occurred about 16 weeks following the injection of STZ. The maximum increases in amine concentrations occurred in the distal segments of the tail artery.

Conclusion: STZ-diabetes is associated with an increase in the concentrations of noradrenaline, dopamine and serotonin in all segments of the tail artery of the rat. These peak between 10 and 20 weeks following the initial treatment.

Biochemical effects of *Citrullus colocynthis* in non-diabetic and diabetic rats.

¹Al-Ghaithi F, ¹El-Ridi M, ²Adeghate E, ¹Ameri MH
¹Department of Biology, Faculty of Science, ²Department of Anatomy, Faculty of Medicine and Health Science, United Arab Emirates University, Al Ain, UAE.

Diabetes mellitus is one of the most common endocrine diseases in the world that affects almost 6% of the world population (1). In UAE many traditional plants are used as anti-diabetic remedies, such as the *Citrullus colocynthis*

(Handal). The aim of this study was to examine the effect of the oral administration of aqueous extract of *Citrullus colocynthis* seeds on biochemical and metabolic parameters of normal and streptozotocin (STZ)-induced diabetic rats. Diabetes mellitus (DM) was induced by a single intraperitoneal (60 mg Kg body wt⁻¹) injection of streptozotocin. Normal and diabetic rats were fed with the plant extract daily by oral intubations for two weeks. Blood sample collected at the beginning and at the end of the experiment for measurement of biochemical parameters. The plasma level of ALT increased significantly after the onset of experimental diabetes. In contrast, the plasma level of ALT decreased significantly after the administration of the plant extract. All of these results raised and confirm a number of interesting issues. Firstly, that STZ has a hepatotoxic effect by increasing the plasma level of ALT and secondly, the aqueous extract of the *C. colocynthis* can ameliorate the toxic effect of STZ in the liver. Moreover, this study suggests that *C. colocynthis* is not toxic at least when given in the higher doses in this study. In diabetic rats there was a significant increase in plasma GGT level. This elevation is an indicator of hepatotoxicity caused by STZ. There were no significant changes in the level of GGT even after treating diabetic rats with low and moderate doses of the aqueous extract of the plant. There was no significant change in the blood level of creatinine, calcium, sodium, phosphorus. It is not known why there were no changes in these biochemical parameters after treatment with the plant extract. It is possible that the concentration of the extract may be too weak to have a significant effect or the tissue or organ damage may be too severe to show any detectable improvement. In conclusion, the results of this study revealed that oral administration of the aqueous extract of the *Citrullus colocynthis* can ameliorate the toxic effect of STZ.

Reference

1. Adeghate E. Diabetes mellitus: multifactorial in aetiology and global in prevalence. Arch. Physiol. Biochem. 109: 197-199, 2001.

Increased Incidence of Diabetic Neuropathy on Smokers: 9 year Follow Up Result of Sheffield Prospective Diabetes Study

S M Rajbhandari (1), LA Scott (2) N D Harris (2) J D Ward (2) & S Tesfaye (2)
Lancashire Teaching Hospital, Chorley & South Ribble, PR7 1PP, (2) Diabetes Research Unit, Royal Hallamshire Hospital, Sheffield S10 2JF, UK.

Introduction: A number of macrovascular risk factors have been associated with the development of diabetic neuropathy (DN), however the relation of smoking to DN has not been clearly established in a prospective study. **Aims:** The aim of Sheffield Prospective Diabetes study was to identify the early abnormalities of clinical, biochemical, neurophysiological and haemorrhological functions for the development of complications of type 1 diabetes. **Materials and Methods:** 66 newly diagnosed diabetes subjects (mean age 31 ± 9 (SD) duration (3 years \pm 2) were identified and followed up for 9 years. They had detailed smoking history

and neurological assessment (symptoms and sign score, nerve conduction, vibration perception threshold, warm thermal discrimination threshold and cardiac autonomic function test) done at baseline and at follow up. Dyck's criteria were used to define DN. 9 subjects who had high c-peptide and 10 subjects who had neuropathy at baseline were excluded from the analysis. **Results:** Out of 40 patients followed up at 9 years 7 (17.5%) developed DN of which 5 (71%) were smokers at baseline ($p < 0.0001$), which was significant after adjusting for the HbA1c. Of the 28 non-smokers at baseline only 2 (7.1%) developed DN. On detail neurophysiological assessment smoking was associated with significant ($p < 0.05$) reduction in sural nerve conduction and rise in vibration perception threshold. Smokers were also likely to develop microalbuminuria ($p < 0.0001$) and there was a trend for the development of retinopathy ($p = 0.07$) on follow up. **Conclusion:** This prospective study confirms that smoking is a significant risk factor for the development of DN and suggests a vascular abnormality as its aetiology. Diabetic subjects who smokes should be actively encouraged to stop this to prevent both macrovascular and microvascular complications.

Protein modifications and apoptosis in Diabetes

Alam Nur-E-Kamal

Department of Pharmacology, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, 675 Hoes Lane, Piscataway, NJ 08854, U.S.A. Phone: (732) 235-0106, Fax: (732) 235-4073, e-mail: nurekasa@umdnj.edu

Appropriate regulation of apoptosis has been hypothesized to play a key role in the development of diabetes, cancer, neuron degeneration, carcinogenesis and aging. Diabetes mellitus is increasing worldwide. It is characterized by insulin resistance and a progressive loss of beta-cell function. A current hypothesis is that hyperglycemia might be responsible for reduction of the mass of beta cells by inducing apoptosis. It is our hypothesis that increased glucose level causes hyperglycation of the receptor proteins, which increase cellular reactive oxygen species (ROS). Increased ROS produces DNA damage signals leading to apoptosis. We have produced a system to investigate the molecular mechanism of ROS-induced DNA damage signaling. We have observed an increase in ROS-dependent protein modification on diabetic and old rats. We have also demonstrated ROS-dependent activation of a DNA damage-signaling pathway that induces apoptosis of mammalian cells in cell culture condition. Nuclear proteins, ATM and p53, are required in transducing DNA damage signals from the nucleus to the mitochondria. The potential role of nuclear-mitochondrial signaling in inducing apoptosis in diabetes will be discussed.

Diabetes-alcohol interaction: The effect of alcohol treatment during pregnancy on fetal development in streptozotocin-induced maternal diabetes in the mouse.

Padmanabhan R and M Shafiulla,
Faculty of Medicine and Health Sciences, UAE University, Al Ain, United Arab Emirates.

Both maternal diabetes mellitus and alcohol consumption during pregnancy have been reported to increase the risk of fetal malformations and intrauterine growth alterations (IUGR) in the offspring. But it is not known if alcohol interacts with maternal diabetes and what the fetal outcome might be if alcohol exposure occurs in diabetic women during pregnancy. Mice were made diabetic by a single dose injection (IP) of streptozotocin (STZ) (200 mg/kg) on gestation day (GD) 2. Those with a blood sugar level of 200 mg/dl or more determined 24 hrs later were regarded as diabetics. They were subsequently injected (IP) on GD 7 or 8 with a single dose of either 0.003ml/g or 0.03ml/g of 25%v/v of ethanol in normal saline or a proportionate volume of saline alone. Fetuses were collected on GD 18. The diabetic animals consumed more food and water, maintained high blood sugar levels throughout pregnancy and gained relatively less body weight than the controls. Both the STZ and STZ plus ethanol groups of fetuses had several craniofacial anomalies such as maxillary-, and mandibular hypoplasia, arched palate and consistent growth retardation. A large number of the diabetic group fetuses had developed a complex holoprosencephaly sequence comprising a proboscis, median eye, mandibular-, maxillary agnathia microstomia/astomia, microglossia, cleft palate, and extreme reduction in anteroposterior diameter of the skull base. There were considerable inter-individual and inter-litter variations in the severity and combinations of these anomalies. Both holoprosencephalic embryos and apparently non-malformed embryos had pronounced thymus hypoplasia and occasional urogenital malformations. The data indicate that both maternal alcohol and diabetes act synergistically in inducing malformations and IUGR in mouse fetuses.

(This work was generously supported by the UAE University)

Ameliorative effects of α -lipoic acid on maternal diabetes-induced growth retardation and malformations in rat fetuses.

MHM AL Ghafli, R Padmanabhan, HH Kataya, B Berg
Faculty of Medicine, UAE University, Al Ain, United Arab Emirates.

Background: Infants of diabetic women have an increased incidence of birth defects than those of control population. The malformations arise early during development and contribute significantly to perinatal mortality and morbidity. Oxidant-antioxidant disequilibrium associated with the production of excessive reactive oxygen species (ROS) in diabetes may be a contributing factor to fetal anomalies. α -Lipoic acid (LA) has been described as an effective antioxidant against a variety of ROS including those associated with diabetes. The objective of the present study was to determine the protective effects if any, of LA against maternal diabetes-related malformations and intrauterine growth retardation (IUGR) in rat fetuses. **Methods:** Pregnant rats were divided into 5 groups: Group I was non-treated. Group II was made diabetic on gestation day (GD) 2 by a single intraperitoneal (IP) injection of

streptozotocin (STZ). Group III was injected with 20mg/kg of LA (IP) daily starting on GD 6 and continued through GD 19. Group IV was administered only Tris buffer on the corresponding days. Group V was STZ-treated but followed by a daily dose of 20mg/kg (IP) of LA starting on GD 6 and continued through GD 19. Fetuses were collected on GD 20. Results and conclusions: Treatment of diabetic rats with LA did not affect their blood sugar levels significantly but improved their body weight gain and reduced their food and water consumption. STZ group had a manifold increase in embryonic death/resorption, IUGR and malformations rate when compared to the control groups. Craniofacial malformations, supernumerary ribs and axial and appendicular skeletal hypoplasia were the major defects of diabetic group. Supplementation with LA significantly reduced the incidence of fetal death, IUGR, malformations, skeletal anomalies and hypoplasia. Considering the antioxidant properties of LA, the preventative effect of LA on diabetes-associated fetal complications suggests that the normal processes of embryo survival, growth and development depend on the availability of antioxidants. Our data also support the hypothesis that ROS are causally related to fetal maldevelopment and IUGR associated with maternal diabetes in the rat.

(This work was generously supported by the UAE University)

Investigations into the membrane interactions of m-calpain, an enzyme implicated in diabetic cataractogenesis

David Andrew Phoenix¹, Sarah Dennison², Fred Harris², Thomas Haub³, Silvia Dante³, Klaus Brandenburg⁴ and Suman Biswas⁵.

¹Deans Office, Faculty of Science, University of Central Lancashire, Preston, UK ² Department of Forensic and Investigative Science, University of Central Lancashire, Preston, UK; ³ Berlin Neutron Scattering Centre, Hahn-Meitner Institute, ⁴ Germany. Forschungsinstitut, Division of Biophysics, Borstel, Germany; ⁵ Department of Ophthalmology, Royal Preston Hospital, Preston, UK.

m-Calpain is a Ca^{2+} - dependent heterodimeric protease, ubiquitous in mammalian cells and recent studies have suggested specific activation of the enzyme in diabetic cataractogenesis. Despite intensive study, the mechanism(s) underlying m-calpain activation are not fully understood: *In vitro* activation of the enzyme requires millimolar levels of Ca^{2+} , far exceeding those found intra-cellularly, and suggesting that *in vivo* activation of m-calpain involves other factors. Lipid has been shown to lower the *in vitro* Ca^{2+} levels needed to activate the enzyme and we have recently predicted that a GTAMRILGGVI segment in domain V of the m-calpain smaller subunit will form a lipid interactive, oblique orientated α -helix. Here we have tested this prediction using VP1, a peptide homologue of the GTAMRILGGVI segment, and lipid assemblies mimetic of naturally occurring membranes. FTIR conformational

analysis showed this peptide to be 80% α -helical in the presence of DMPC/ DMPS (10:1 molar ratio) vesicles. Monolayer studies showed VP1 to induce surface pressure changes of 4 mN M^{-1} in POPC / POPS (10:1 molar ratio) monolayers, suggesting interaction with the lipid monolayer acyl chain region. Moreover, neutron diffraction studies showed VP1 to be localized to the hydrophobic core of model POPC / POPS (10:1 molar ratio) bilayer structures, consistent with the oblique membrane penetration predicted for the peptide. In combination, these results suggest that interactions between domain V of m-calpain and lipid / the membrane may play a role in the activation of the enzyme.

Voltage dependence of contraction in STZ-induced cardiac myocytes

N.K.Bracken, A.J. Woodall and J.Singh) Department of Biological Sciences, University of Central Lancashire, Preston

It has been reported that diabetic cardiomyopathy is associated with increased mortality and morbidity (Schaffer (1991) *Mol Cell Biochem* 107, 1-20). Moreover, it has also been reported that hearts from animal models with (streptozotocin) STZ-induced type-1 diabetes have altered contractility (Choi *et al.* (2002) *Am J Physiol* 283, H1398-H1408). The aim of this study was to ascertain if membrane voltage contributes to changes in contraction within the diabetic heart. Diabetes was induced in male Wistar rats by i.p injection of STZ. Ventricular myocytes were isolated according to previously described techniques (Frampton *et al.* (1991). *J Physiol* 437, 351-375). Patch-clamp recordings were made in whole cell, voltage clamp mode using after correcting for membrane capacitance, series resistance. From a holding potential of -40 mV , test pulses were applied at potentials between -30 and $+60 \text{ mV}$ in 10 mV increments (Howarth FC & Levi AJ (1998). *Pflugers Arch* 435, 687-698). $I_{\text{Ca,L}}$ and contraction was measured simultaneously using a video edge detection system. STZ-induced diabetes resulted in a significant reduction in body and heart weight. Whole blood glucose and plasma osmolarity were significantly decreased, while, plasma insulin was significantly decreased. Membrane capacitance (pF) was not significantly altered between control ($102.7 \pm 5.64 \text{ pF}$, (n=9)) and STZ-induced diabetic myocytes ($100.6 \pm 2.09 \text{ pF}$, (n=9)). The $I_{\text{Ca,L}}$ was significantly ($P < 0.05$) reduced throughout voltage ranges (0 mV and $+40 \text{ mV}$) in STZ-induced myocytes when compared to age-matched controls. Moreover, contraction was significantly ($P < 0.05$) greater in control myocytes compared to STZ-induced myocytes at all test potentials, however there were no significant differences in the time to peak of $I_{\text{Ca,L}}$ in control and STZ-treated myocytes. A decrease in contraction is indicative of either reduced SR Ca^{2+} release or decreased myofilament sensitivity to Ca^{2+} . It is suggested that in the diabetic heart, a reduction peak $I_{\text{Ca,L}}$ leads to decreased SR Ca^{2+} release and a reduction in contraction.

This work was supported by the British Heart foundation

Effects of acidosis on contraction and intracellular Ca^{2+} in ventricular myocytes from streptozotocin – induced diabetic rat

Singh J, Qureshi MA, Howarth FC
Department of Physiology, Faculty of Medicine and Health Sciences, UAE University, Al Ain, UAE

The concentration of acetoacetic acid, β -hydroxybutyric acid and acetone in blood may rise to very high levels in diabetic patients and cause metabolic acidosis. The aim of this study was to investigate the acute effects of acidosis on ventricular myocyte contraction and Ca^{2+} transport in streptozotocin (STZ) - induced diabetic rats. Diabetes was induced in rats by i.p. injection of STZ (60 mg kg^{-1}). Experiments were performed at 35-36 °C in electrically stimulated (1 Hz) ventricular myocytes from control and diabetic rats at 8-12 weeks after administration of treatment. Characteristics of shortening were measured with a video edge detector (VED-114, Crystal Biotech, USA). Intracellular Ca^{2+} was measured in myocytes loaded with fura-2/AM. Electrically stimulated myocytes were exposed to normal Tyrode solution pH adjusted to either 7.4 (NT) or 6.4 (acid NT). The general characteristics of STZ-induced diabetes included significant reductions in body and heart weight and a 4-fold increase in blood glucose. Time to peak shortening was significantly prolonged in myocytes from diabetic compared to control rats. Change of external pH from 7.4 to pH 6.4 significantly reduced the amplitude of shortening in myocytes from control and diabetic rats however, the magnitude of the negative inotropic effects of acid NT were not additionally altered by STZ-treatment. The amplitude of the Ca^{2+} transient was also significantly reduced by acid NT but the magnitude of the response was not additionally altered by STZ-treatment. The acute effects of exposure to acid NT on myocyte shortening and Ca^{2+} transient were not significantly altered by STZ-induced diabetes.

Does QT dispersion predict the future development of cardiac autonomic neuropathy in type 1 diabetes?

S M Rajbhandari (1), L Yeoh (2) N D Harris (2) J D Ward (2) & S Tesfaye (2)
(1) Lancashire Teaching Hospital, Chorley & South Ribble, PR7 1PP, (2) Diabetes Research Unit, Royal Hallamshire Hospital, Sheffield S10 2JF, UK.

Background: Increased QT dispersion (QTD), the marker of cardiac electric heterogeneity has been recognised as a marker of sudden cardiac death. In diabetes QTD has been suggested to be related to cardiac autonomic neuropathy. However there has been no prospective study to show if increased QTD leads to future development of cardiac autonomic neuropathy. **Aim of study:** The aim of this study was to analyse if increased QTD at baseline predicts the future development of cardiac autonomic neuropathy. **Methods:** We analysed QTD of 36 subjects who had newly diagnosed type 1 diabetes and had ECG performed and followed up for 9 years. Cardiac autonomic function

tests were measured using O'Brien protocol and QTD measured from the rate corrected QT interval in ECG after magnification. The difference between the longest and shortest QTc interval was QTD. The subjects were divided into 2 groups by the median value of 71.4ms. **Results:** There was no difference in the age, sex, HbA1c and baseline neurophysiological parameters between these two groups. After 9 years of follow up there was significant difference only in the RR intervals at rest (1.24 ± 0.09 vs 1.18 ± 0.08 sec ($p=0.03$) but not during deep breathing (1.29 ± 0.13 vs 1.35 ± 0.16 sec ($p=0.18$) with Valsalva (1.71 ± 0.37 vs 1.81 ± 0.45 sec ($p=0.42$) or on standing (1.47 ± 0.25 vs 1.38 ± 0.20 sec ($p=0.35$). There were no difference in other neurophysiological parameters between these two groups. **Discussion:** Our data suggest that there may not be any relation between QT dispersion and development of cardiac autonomic neuropathy.

Risk factors other than neuropathy cause increased prevalence of high risk feet in newly diagnosed elderly type 2 diabetes subjects

H Rathur(1), C Betts(2), S M Rajbhandari(1)
(1)Lancashire Teaching Hospital, Chorley & South Ribble PR7 1PP, (2)Dept of Podiatry, Chorley & South Ribble PCT

Background: Chronic complications are often present by the time of diagnosis of type 2 diabetes (T2D). However, there is paucity of data about various risk factors of foot ulceration such as ischaemia, deformity, poor vision etc in T2D at the time of diagnosis. Similarly there is little information available on characteristics of newly diagnosed T2D subjects who are at high risk of developing diabetic foot ulcers. **Aims of Study:** The aim of this study was to analyse various risk factors for foot ulceration present in a group of newly diagnosed T2D and to identify the subgroup who are at high risk of developing diabetic foot ulcers. **Subjects and Method:** We have a podiatrist led community diabetic foot screening programme and all newly diagnosed T2D subjects are screened within 3 months of diagnosis. We analysed foot screening data of 292 newly diagnosed T2D subjects and classified them into high, medium and low risk categories depending upon previous foot ulcers, neuropathy, ischaemia, deformity and vision. **Results:** History of ulcers were present in 4.1%, impaired 10gram monofilament sensation in 8.2%, impaired pin prick sensation in 20.9% subjects, ischaemia in 26.7%, foot deformity in 34.6% and poor vision in 11.1%. Increasing age was significantly ($p=0.005$) related to 'at risk feet' with 15% [mean age 66.7 ± 11.9 years] having high risk, 29.5% [mean age 66.4 ± 10.6 years] having medium risk and 55.5% [mean age 61.6 ± 11.1 years] having low risk feet. Age was positively associated with ischaemia ($p=0.018$), deformity ($p=0.002$), visual problem ($P=0.002$) and living alone ($P=0.001$) but not with previous ulcers or neuropathy. There were no differences in the risk factors between males and females. **Conclusions:** Our data confirms the need for foot screening of all newly diagnosed T2D subjects with special attention to elderly.

To determine incidence of risk factors and complications in newly-referred diabetics in the hospital outpatient clinic.

S Rajpara, KE Imtiaz
Department of Diabetes and Endocrinology, Lancashire Teaching Hospital, Chorley, UK

A retrospective analysis of 58 diabetics (37 males, 21 females) referred to the hospital outpatient diabetes clinic from August 2001 to July 2002 was performed. Number of type 1 and type 2 diabetics were 9(15.5%) and 49(84.5%) respectively. Mean age was (type 1: 35.4, type 2, 60.3; $p=0.0001$) and duration of diabetes (type 1, 9.12 ; type 2, 6.22 ; $p=0.0269$). 24% of diabetics were current smokers while 10% were ex smokers. 82% among this group were overweight while 55 % (24/44) were obese. 65% of patients had HbA1c >7 % on initial referral. Mean BP was 140.7/78.9 (type 1 , 125.8/73.2 ; type 2 , 143.4/79.9 ; $p=0.0003$) ; mean cholesterol/HDL ratio: 4.52 (type 1, 4.27 ; type 2 , 4.56 ; $p=0.29$) and mean triglycerides: 2.3 (type 1 , 1.73 ; type 2 , 2.39 ; $p=0.1$). The incidence of risk factors and complications in diabetics was as follows:

Microalbuminuria (52%), Hypertension (48%), ischaemic Heart Disease (IHD, 22%), peripheral neuropathy (14%), cerebrovascular Disease (9%), retinopathy (7%) and foot ulcers(2%). Only 1 patient had raised serum creatinine (>150 micromoles per litre) on initial referral.

Type 1 diabetic patients were younger with longer duration of diabetes, while type 2 had significantly high blood pressure. 50 % of all patients were obese and there was high incidence of cardiovascular risk factors, e.g. microalbuminuria , hypertension, and ischaemic heart disease.

Audit of risk factor modification and management of complications in newly referred diabetic patients to the hospital outpatients' clinic.

S Rajpara, KE Imtiaz.
Department of Diabetes and Endocrinology, Lancashire Teaching Hospital, Chorley, UK

A retrospective analysis of 58 diabetics (37 males, 21 females) referred to hospital outpatient diabetes clinic from August 2001 to July 2002, was performed. Number of type 1 and type 2 diabetics were 9 (15.5%) and 49 (84.5%) respectively. All type 1 were on insulin while 51% of type 2 were on single oral hypoglycaemic drug (OHD) , 24% on diet alone, 20 % on 2 or more OHD, 6% on combined OHD and insulin. 48% of these patients were hypertensive: 64% receiving ACE inhibitors, 39% calcium channel blocker, 32% beta blockers, 29% diuretics, 21% Angiotensin II receptor blocker, 4% alpha blockers and 1.9% centrally acting drugs. Most of these patients required combined antihypertensive treatment: 50% (2 drugs) , 36.2% (3 drugs) and 13.4% (4 , >4). 13/58 (22%) had raised cholesterol/HDL ratio > 5 with only 31% on statins. Out of 22 (37.5%) patients with high microalbumin/creatinine ratio, only 12/22 (55%) were on ACE inhibitors. Only 1 patient had serum creatinine >150

micromol per litre and was referred to nephrologist. 85% of patients with ischaemic heart disease and 80% with cerebrovascular disease were on antiplatelet therapy respectively. 7% of patients in our group had retinopathy and all were referred to the ophthalmologist. Only 1 patient had foot ulcer and was referred to the ulcer clinic.

Only one-third of eligible patients in our group were receiving cholesterol lowering therapy, and only >50 % of these were on ACE inhibitors for microalbuminuria. Most diabetic patients require a combination of drugs to achieve the current target for glycaemia, lipids and blood pressure. Aggressive risk factor management is warranted in all diabetic patients.

Increased Platelet Activation Predicts the Development of Diabetic Neuropathy

S M Rajbhandari (1), LA Scott (2) N D Harris (2) J D Ward (2) & S Tesfaye (2)
(1) Lancashire Teaching Hospital, Chorley & South Ribble, PR7 1PP
(2) Diabetes Research Unit, Royal Hallamshire Hospital, Sheffield S10 2JF, UK.

Introduction: The exact pathogenic mechanism of diabetic neuropathy (DN) is not fully understood. Cross-sectional studies have shown an association between platelet activation (PA) and diabetic retinopathy and nephropathy. However, the relationship of PA to the development of diabetic complications including neuropathy has not been studied prospectively. **Aims:** The aim of this study was to examine the association between PA and development of microvascular complications including DN in a prospective study in a cohort of newly diagnosed type 1 diabetic subjects. **Methods:** We followed 44 newly diagnosed type 1 diabetic subjects (age 31 +/- 9 [SD] years; duration 3 +/- 2 years) who underwent detailed neurological assessment and plasma β -thromboglobulin (BTG) to assess PA, at baseline, 3 and 9 years. Using baseline BTG levels of 25ng/ml as a cut-off point, subjects were divided into two groups (18 low & 26 high). **Results:** DN defined as per Dyck's criteria was significantly more prevalent in the high BTG group at 9 years (11.14 % Vs 38.5%; $p=0.003$). There was no difference in peroneal nerve conduction velocity between the two groups at baseline and 3 years, but it was significantly reduced by 9 years in the high BTG group even after the data was adjusted for HbA1c (43.1 +/- 4.5 Vs 37.6 +/- 5.7 m/sec; $p=0.035$). **Conclusions:** This prospective study suggests that platelet activation may have a role in the development of DN and hence support the vascular dysfunction as a mechanism of DN.

Orally Administered Tryptophan and Experimental Type-2 Diabetes.

Tormo MA, Romero de Tejada A, Morales I, Paredes S, Sánchez S, Barriga C, Hernández R
Departamento Fisiología. Facultad de Medicina. Universidad de Extremadura. 06071 Badajoz. SPAIN

Introduction: There is a link between diabetes and

oxidative stress. Hyperglycaemia leads to free radical generation and alterations of endogenous antioxidants. Our aim is to study the effect of orally administered L-tryptophan, the melatonin precursor, an endogenous antioxidant, on circulating levels of glycaemia, insulin, and melatonin, and on the superoxide dismutase and catalase antioxidant systems in non-diabetic (ND) and type-2 diabetic (neonatal diabetes model: n5-STZ) male Wistar rats. **Materials and Methods:** At 19:30 every day for 15 days L-tryptophan (TRP: 125 mg/kg b. w.) was administered orally. At 09:00 every two days the glycaemia was measured and every day the intake of food and water was recorded. At the beginning and end of treatment (at 09:00; 21:00; 02:00) plasma insulin and melatonin levels were measured by radioimmunoassay, and (at 09:00) the enzymatic activities of catalase (mg/g haemoglobin) and superoxide dismutase (SOD, UI/g haemoglobin) in erythrocytes by spectrophotometry. The results are expressed as mean \pm SEM (n=6 for each group). **Results:** Glycaemia values (mmol/L) were greater (p<0.01) in n5-STZ rats (day 0: 18.74 \pm 2.32 and day 15: 22.01 \pm 2.99) than in ND rats (5.16 \pm 0.15 and day 15: 5.49 \pm 0.32), while insulin levels were lower (p<0.05) at all times studied. These parameters were not altered by the TRP administration. Melatonin levels (pg/ml) at 02:00 were lower in n5-STZ (22.25 \pm 11.22) than in ND rats (63.17 \pm 19.38; p<0.05). The TRP administration did not modify the circulating melatonin levels in ND rats, but raised (p<0.01) the levels at 02:00 in treated n5-STZ rats (56.66 \pm 7.17) vs the untreated group (8.87 \pm 3.29). In ND rats after TRP administration there was a decline in catalase activity (day 0: 20.61 \pm 0.93; day 15: 17.57 \pm 1.16; (p<0.05) while in n5-STZ rats there was a rise (day 0: 18.69 \pm 1.22; day 15: 27.76 \pm 2.37; p<0.01). After TRP administration there were no significant changes in SOD activity or reduction in water intake ml/day in either ND or n5-STZ rats, but there was increased food intake (g/day) in treated n5-STZ (48.95 \pm 0.79) vs untreated n5-STZ rats (34.62 \pm 1.05; p<0.01). **Conclusions:** The oral administration of L-tryptophan: 1.-did not modify glycaemia or insulinaemia levels; 2.- raised melatonin levels in diabetic rats at 02:00; 3.- lowered catalase activity in ND rats but raised it in n5-STZ rats; 4.-increased food intake in n5-STZ rats. This work was financed by the Consejería de Sanidad y Consumo, Junta de Extremadura, Spain.

Beneficial effects of *Momordica Charantia* fruit juice in diabetes mellitus

J.Singh*, A. Sharma, E. Adeghate, I. Ahmed and E. Cummings. Department of Human Anatomy, FMHS, United Arab Emirates University, Al-Ain, UAE and *Department of Biological Sciences, University of Central Lancashire, Preston, England.

Diabetes Mellitus (DM) is a major global health problem and diet and exercise play major roles in the management of DM. Prior to insulin therapy, the main form of treatment was dietary measures, including the use of traditional medicines derived from plants (Platel & Srinivasan, *Nahrung* 41(2), 68-74, 1997). The fruit juice of one such

plant is *M. charantia* (family: Cucurbitaceae), which is widely used to treat DM. This study investigates the beneficial effect of *M. charantia* fruit juice in diabetic treated rats. In addition, the effect of the juice on glucose transport in L6 muscle cells was also investigated for comparison. Rats were rendered diabetic by a single (I.P) injection (60 mg (kg⁻¹ body wt) of streptozotocin (Sharma *et al*, *Int. J. Diabetes* 4, 29-38, 1996). One week after injection, treated animals were fed with the juice (10 ml kg⁻¹) daily for 2-3 months. Body weight, blood pressure, glucose levels were measured routinely. Morphological studies were undertaken to confirm diabetes and membrane vesicles from jejunum were isolated for the measurement of glucose uptake. Blood glucose concentrations in control, diabetic and treated rats ranged from 55 – 64, 414 – 509 and 350 – 400 mg dl⁻¹ (n=6), respectively. Insulin concentrations in the plasma of normal, diabetic and diabetic-treated animals were 5.9 \pm 0.25, 0.56 U \pm 0.37 and 1.14 \pm 0.04 ng ml⁻¹ (n=5 animals), respectively. Similarly, the number (mean \pm SEM, n=5) of insulin-positive cells per islet was 22.97 \pm 3.01 (n=5), 10.97 \pm 1.23 (n=5) and 16.61 \pm 1.53 (n=5) in control, diabetic and treated diabetic rats, respectively. Systolic blood pressure was 58 \pm 8.30, 122.8 \pm 19.99 and 91.2 \pm 14.4 m Hg in normal, diabetic and treated rats. The sodium dependent glucose uptake in brush border membrane vesicles isolated from normal, diabetic and diabetic treated rat jejunum was (mean \pm SEM) 428.7 \pm 136 (n=6), 1802.3 \pm 423 (n=6) and 368.6 \pm 69.2 pmol (mg protein)⁻¹, respectively. In L6 muscle cells, either insulin or physiological doses 1-10 μ g ml⁻¹ of *M. charantia* fruit juice evoked significant (P<0.05) increases in glucose and amino acid uptakes after 1 – 6 hours of incubation compared with control. The results indicate that *M. charantia* can have marked beneficial effects in the treatment of DM.

Secretagogue effect of insulin on pancreatic juice secretion in the anaesthetized normal and diabetic rats

M.D. Yago*, J. Singh, M. Mañas*, E. Martinez-Victoria* and R. Patel. Department of Biological Sciences, University of Central Lancashire, Preston, UK and *Institute of Nutrition, University of Granada, Granada, Spain

Insulin is a major metabolic hormone which can regulate glucose metabolism. In the *in vitro* pancreas, insulin has little or no effect on digestive enzyme secretion. In contrast, it can potentiate the secretagogue effects of ACh and CCK-8 (Singh *et al* (1992) *Exp. Physiol.* 77, 191-202). To date, no study has yet investigated the secretagogue effect of insulin in the anaesthetized normal and diabetic rat preparation. Animals were rendered diabetic by a single injection of streptozotocin (STZ, 60 mg kg⁻¹ I.P.). Age-matched controls were injected with an equal volume of citrate buffer. The rats were tested for hyperglycaemia 4 days after STZ injection and 7 weeks later when they were used for the experiments. Following general anaesthesia (1 g kg⁻¹ urethane I.P.), laparotomy was performed and the pancreatic duct cannulated for the collection of juice using established methods (Singh *et al*, 1992). After the experiments, rats were humanely killed by urethane

overdose. Administration of insulin (1 IU, I.P.) resulted in time dependent and significant ($P < 0.05$) increases in pancreatic flow rate, protein output and amylase secretion in control animals compared to basal secretory parameters. Maximal effects occurred after 40 min of insulin administration. The action of insulin was also associated with a time dependent decrease in blood glucose levels (152.7 ± 16.9 mg dl⁻¹ ($n=6$) prior to insulin and 42.0 ± 8.4 mg dl⁻¹ ($n=4$) 100 min later). In diabetic rats insulin (4 IU, I.P.) evoked delayed increases in flow, protein output and amylase secretion with maximal responses occurring after 120 min of insulin administration. Blood glucose level was 467.6 ± 14.0 mg dl⁻¹ ($n=10$) prior to insulin and this value decreased slowly to around 386.6 ± 43.6 mg dl⁻¹ ($n=7$) at 120 min post-insulin. The results indicate that insulin can evoke marked pancreatic secretagogue effects and stimulate glucose metabolism in the healthy rats compared to reduced responses in diabetic animals.

***Mormordica charantia* fruit juice stimulates glucose and amino acid uptakes in L6 myotubes.**

E. Cummings, H. Hundal*, H. Wackerhage*, N. Woods and J. Singh Department of Biological Sciences, University of Central Lancashire, Preston and *Department of Physiology, MSI/WTB Complex, University of Dundee, Dundee

The fruit of *Mormordica Charantia* (family: Cucurbitaceae) is used widely as a hypoglycaemic agent to treat diabetes mellitus (DM). The mechanism of the hypoglycaemic action of *M. charantia* *in vitro* is not fully understood. This study investigated the effect of *M. Charantia* juice on either ³H-deoxy-D-glucose or N-methyl-amino-*a*-isobutyric acid (¹⁴C-Me-ATB) uptake in L6 rat muscle cells cultured to the myotube stage. The fresh juice was centrifuged at 5000 r.p.m and the supernatant lyophilised. L6 myotubes were incubated with different concentrations of either insulin (100 nM), the juice (1-10 µg ml⁻¹) or wortmannin (100 nM) over a period of 1-6 hours. Results were expressed as pmol min⁻¹ (mg cell protein)⁻¹, $n=6-8$ for each value. Basal ³H-deoxy-D-glucose and ¹⁴C-Me-ATB uptakes by L6 myotubes after 1h of incubation were (means ± SEM) 32.14 ± 1.34 and 13.48 ± 1.86 , respectively. Incubation of L6 myotubes with 100 nM insulin for 1 h resulted in significant (ANOVA, $P < 0.05$) increases in ³H-deoxy-D-glucose and ¹⁴C-Me-ATB uptakes. Typically, ³H-deoxy-D-glucose and ¹⁴C-Me-ATB uptakes in the presence of insulin were 58.57 ± 4.49 and 29.52 ± 3.41 , respectively. Incubation of L6 myotubes with three different concentrations (1, 5 and 10 µg ml⁻¹) of the lyophilised juice resulted in time-dependent increases in ³H-deoxy-D-glucose and ¹⁴C-Me-ATB uptakes, with maximal uptakes occurring at a concentration of 5 µg ml⁻¹. Incubation of either insulin or the juice in the presence of wortmannin (a phosphatidyl inositol 3-kinase inhibitor) resulted in a marked inhibition of ³H-deoxy-D-glucose by L6 myotubes compared to the uptake obtained with either insulin or the juice alone. The results indicate that *M. charantia* fruit juice is acting like insulin to exert its hypoglycaemic effect.

Role of phospholipid signaling in diabetes-induced defective cardiomyocyte contractile activity.

Paramjit S. Tappia.

Institute of Cardiovascular Sciences, St. Boniface Hospital Research Centre and the Department of Human Nutritional Sciences, University of Manitoba, Winnipeg, Canada.

This study was conducted to investigate the effects of phosphatidic acid (PA), a known inotropic agent, on Ca²⁺ transients and contractile activity of cardiomyocytes isolated from chronic streptozotocin-induced diabetic rats. In control cells, PA induced a significant increase in active cell shortening (25%) and Ca²⁺ transients (40%). Maximal effects were found with 25µM PA. Inhibition of phospholipase C (PLC) with NCDC, blocked the positive inotropic action induced by PA. Consistent with this effect, PA increased inositol 1,4,5-trisphosphate (IP₃) generation in the control cardiomyocytes. Furthermore, the PA-induced increase in contractile activity was attenuated by treatment of cardiomyocytes with thapsigargin as well as with nifedipine. Conversely, in cardiomyocytes from diabetic rats, PA-induced a 25% decrease in active cell shortening and no significant effect on Ca²⁺ transients. Basal and PA induced IP₃ generation in diabetic rat cardiomyocytes was 3 fold lower as compared to control cells, which was associated with an impaired sarcolemmal membrane PLC δ1 activity as well as its attenuated response to PA. Insulin treatment of the diabetic animals resulted in a partial recovery of PA responses. Our results, therefore, identify an important defect in the PA-PLC signaling pathway in diabetic rat cardiomyocytes, which may have implications for heart dysfunction during diabetes.

Mechanism of pancreatic insufficiency in streptozotocin-induced diabetes mellitus.

R. Patel*, J. Singh*, E. Martinez-Victoria#, M. Mañas# and M.D. Yago#.

*Department of Biological Sciences, University of Central Lancashire, Preston and #Institute of Nutrition and Food Technology, University of Granada, Granada, Spain.

Exocrine pancreatic insufficiency is a major long term complication in diabetes mellitus and very little is known about the mechanism of this symptom. This study investigated the effects of cholecystokinin-octapeptide (CCK-8) on pancreatic juice flow and its contents, and on cytosolic calcium (Ca²⁺) and magnesium (Mg²⁺) levels in the diabetic rats compared to age-matched control. Animals were rendered diabetic by a single injection of streptozotocin (60 mg kg⁻¹, I.P). Age-matched control rats obtained an equivalent volume of citrate buffer. Seven weeks later, animals were either anaesthetized (1g kg⁻¹ urethane; I.P) for the measurement of pancreatic juice flow or humanely killed and the pancreas isolated for the measurements of cytosolic Ca²⁺ and Mg²⁺ levels. Non-fasting blood glucose levels in control and diabetic rats were 92.40 ± 2.42 mg dl⁻¹ ($n=8$) and >500 mg dl⁻¹ ($n=10$), respectively. Resting (basal) pancreatic juice flow in

control and diabetic anaesthetized rats was $0.49 \pm 0.04 \text{ ul min}^{-1}$ ($n=10$) and $1.17 \pm 0.09 \text{ ul min}^{-1}$ ($n=11$). CCK-8 infusion resulted in a significant ($P<0.05$) increase in pancreatic juice flow in control animals compared to a much larger increase in diabetic rats. In contrast, CCK-8 evoked significant ($P<0.05$) increases in protein output and amylase secretion in control rats compared to much reduced responses in diabetic animals. Basal $[\text{Ca}^{2+}]_i$ in control and diabetic fura-2-loaded acinar cells was $109.40 \pm 15.41 \text{ nM}$ ($n=15$) and $130.62 \pm 17.66 \text{ nM}$ ($n=8$), respectively. CCK-8 (10^{-8} M) induced a peak response of $436.55 \pm 36.54 \text{ nM}$ ($n=15$) and $409.31 \pm 34.64 \text{ nM}$ ($n=8$) in control and diabetic cells. Basal $[\text{Mg}^{2+}]_i$ in control and diabetic magfura-2-loaded acinar cells was $1.00 \pm 0.06 \text{ nM}$ ($n=18$) and $0.88 \pm 0.04 \text{ nM}$ ($n=10$). In the presence of CCK-8 (10^{-8}) $[\text{Mg}^{2+}]_i$ in control and diabetic cells was $0.80 \pm 0.05 \text{ nM}$ ($n=18$) and $0.60 \pm 0.02 \text{ nM}$ ($n=10$), respectively. The results indicate that diabetes-induced pancreatic insufficiency may be associated with derangements in cellular Ca^{2+} and Mg^{2+} homeostasis.

Comparison of diabetes care between category B and category C prison

K Wright, F Rathur, S M Rajbhandari
Lancashire Teaching Hospital, Chorley, PR7 1PP, UK

Introduction: The management of diabetes in prison can be difficult, as inmates have limited freedom on diet, exercise and access to external health care. We extended care to inmates by regular visit of diabetes specialist nurse to local prisons and train prison nurses. To assess the effect, we analysed the diabetes annual review prisoners received. **Aims:** The aims of this study was to find out the quality of diabetes annual review care inmates received in prison and to compare the care between two prisons. **Results:** Data on 13 male subjects [mean age \pm (SD) 50.2 ± 14.3 years] in category B prison were compared with 8 male subjects [mean age 53 ± 9.5 years] in category C prison. In B prison 46.1% subjects were on insulin, 38.5% on oral hypoglycaemics and 15.4% on diet alone and in C prison it was 12.5%, 75% and 12.5%. Screening for retinopathy and feet were arranged in 100% and 46.1% in B prison and 87.5% and 62.5% in C prison. Blood pressure control in both prisons was similar and none had developed nephropathy. There were no differences in HbA1c (7.97 ± 2.26 vs 7.7 ± 1.21 ; $p=0.9$) or cholesterol (5.05 ± 1.07 vs 5.52 ± 1.5 ; $p=0.9$) between these prisons. **Conclusion:** Our data suggest that good diabetes care can be provided to prison inmates and the category of prison does not affect this.

Halothane inhibits contraction and calcium mobilization in streptozotocin-induced diabetic rat ventricular myocytes.

A.Woodall*, J.Singh*, F.C. Howarth# & N.K. Bracken* .
*Department of Biological Sciences, University of Central Lancashire, Preston and #Department of Physiology, FMHS, United Arab Emirates University, Al Ain, UAE

Defective cardiac function is a frequent complication of human diabetes and volatile anaesthetics such as halothane can exert a potent negative inotropic effect in the heart. This study investigated the effects of halothane (0.6 mM) on contraction and on calcium transport in ventricular myocytes from streptozotocin (STZ)-induced diabetic rats compared with age-matched controls. STZ (60 mg kg^{-1}) was administered intraperitoneally (I.P) to adult male Wistar rats, which were humanely killed 8 weeks later. Both contraction and Ca^{2+} transport were measured by established techniques (Howarth, F.C & Levi, A.J. (1998) *Pfluges Arch.* **435**, 687-698). The amplitude of contraction as a percentage of resting cell length was significantly ($P<0.01$) altered in STZ-induced diabetic myocytes ($6.8 \pm 0.5\%$, $n=32$) than that of control ($4.1 \pm 1.04\%$, $n=27$). The t_{pk} of contraction was significantly ($P<0.01$) longer in diabetic myocytes ($164.1 \pm 7.4 \text{ ms}$, $n=30$ Vs. $132.3 \pm 5.9 \text{ ms}$, $n=27$) compared to control. Halothane evoked significant ($P<0.05$) reductions in the amplitude of contraction in control and STZ-induced myocytes compared to the responses in the absence of halothane for each. The amplitude of $I_{\text{Ca,L}}$ was significantly ($P<0.05$) decreased in diabetic myocytes ($-0.32 \pm 0.03 \text{ nA}$, $n=7$ Vs. $0.66 \pm 0.05 \text{ nA}$, $n=8$) compared to control at +10 mV, respectively. Halothane also reduced the peak $I_{\text{Ca,L}}$ to levels in control myocytes similar to the reduction in $I_{\text{Ca,L}}$ seen in diabetic cells. Basal cytosolic Ca^{2+} (ratio units) was significantly ($P<0.01$) increased in diabetic myocytes compared to control (0.599 ± 0.009 , $n=23$, Vs. 0.521 ± 0.012 ratio units, $n=23$), respectively. Electrically stimulated cardiac myocytes (1 Hz) induced Ca^{2+} transients that had a longer time from the peak (t_{pk}) of Ca^{2+} transient to half decay ($t_{1/2 \text{ relax}}$). Halothane, evoked significantly ($P<0.05$) decreases in the Ca^{2+} transients in control and STZ-induced myocytes compared to the responses obtained in the absence of halothane. The results indicate that halothane is exerting its negative inotropic effects by reducing cellular Ca^{2+} homeostasis.

The outcome of abnormal glucose tolerance states in Central Lancashire

Abdul Lakhdar,
Lancashire Teaching Hospitals NHS Trust, Preston, UK.

The diagnostic criteria for diabetes mellitus and glucose intolerance has recently been redefined. The American Diabetic Association (ADA) advocated the use of fasting plasma glucose (FPG) alone, defining diabetes by a fasting plasma glucose concentration of 7.0 mmol/l and de-emphasising the oral glucose tolerance test (OGTT). The WHO new criteria coincided with the ADA recommendation of the use of OGTT if the casual plasma glucose concentration is in the uncertain range of 5.5-11.1 mmol/l. The OGTT identifies individuals who have a diabetic post-challenge blood glucose, but a non-diabetic fasting value. Previous epidemiological studies have shown a high prevalence of isolated post-challenge hyperglycaemia in association with increased mortality risk.

We studied 2769 serial subjects referred with suspected diabetes mellitus (median age 35 years, range 15-94 years) referred over a 3 year period to the Royal Preston Hospital from primary care with suspected diabetes and subjected to a glucose tolerance test.

The prevalence of diabetes was similar according to WHO-1985 and ADA-1997 criteria at 11%. The rate of impaired glucose homeostasis was 12% according to the WHO-1985 and 10% according to the ADA-1997.

906 (33%) individuals showed misclassification. Out of 298 (11%) diabetic individuals by WHO-1985 and 295 (11%) by ADA-1997, only 198 (7%) were diabetic by both criteria. Out of 335 (12%) impaired glucose intolerant individuals by WHO-1985 and 273 (10%) impaired fasting glucose individuals by ADA-1997 criteria, only 97 (4%) had impaired glucose homeostasis by both criteria. 203 (7%) individuals had abnormal glucose tolerance by WHO-1985 criteria (168 IGT and 35 DM), had NFG by ADA-1997.

Although the ADA-1997 and WHO-1985 criteria for the diagnosis of diabetes gave a similar rate of diabetes and impaired glucose homeostasis, a substantial misclassification of individuals results by comparing the two criteria. Of most concern is the ADA-1997 criteria misses 32% of individuals diagnosed with abnormal glucose tolerance according to 2-H PG level. They would have been missed by a screening strategy based on a single fasting plasma glucose alone.

The new WHO criteria identified all individuals with abnormal glucose homeostasis identified by WHO-1985 or ADA-1997 criteria. However, compared with WHO-1985, reclassification occurred in 208 individuals.

1471 patients of the original cohort were followed up for a median time of 5 years (maximum 8 years) to study mortality and glucose tolerance. Information about survival and the cause of death was obtained from the NHS central register (The Office for National Statistics). 42% of this selected group were males. The median age (at time of OGTT) is 51.5 years. The females tend to be younger than the males with a median age of 42.1 years compared to 56.4 years. Out of 1471 patients, 101 (7.4%) died. In 47 patients (3.2%) death was caused by CVD. In total, 46% had an abnormal glucose level. Among those with higher glucose levels, IGT and CH (compared fasting and post-challenge hyperglycaemia) are the largest groups with 14% and 13% respectively. They are followed by IFH (isolated fasting hyperglycaemia), IPH (isolated post-challenge hyperglycaemia) and IFG with about 6% each.

Effect of impaired glucose tolerance and diabetes on mortality

The normal and impaired glucose tolerance groups have similar survival rates within each age/gender group.

Whereas one would expect a worse outcome in the diabetes patients, the survival in older men (older than 60 years) with DM seems to be better than in the older men with NGT or IGT. In contrast, the outcome seems to be worse for diabetic women than non-diabetic. The women survival is better than for men.

Effect of fasting and post-challenge hyperglycaemia on mortality

The reduced mortality in older male diabetic patients compared to those with normal or impaired glucose tolerance is apparent in all 3 abnormal glucose tolerance groups (IFH, IPH and CH) compared to older male patients with normal or impaired glucose tolerance. In women older than 60 years, the increased mortality in the older diabetic patients is mainly in the IPH group. Survival of older women appeared to be similar in all other groups, while in younger women there are only very few events in total.

The survival for diabetic older men appears to be better than for older men with normal or impaired glucose tolerance. Potential explanations for this phenomena should be explored. Apart from men older than 60 years, diabetes is a risk factor for all cause mortality with a hazard ratio of about 2 and for CVD mortality with a hazard ratio of about 4. In women, IPH is associated with a significant increase in all cause mortality.

These results have implications for the use of fasting hyperglycaemia as an acceptable screening test for diabetes.

The modern management of glycaemia in type 2 diabetes and the need to treat to target

Abdul Lakhdar,
Lancashire Teaching Hospitals NHS Trust, Preston, UK.

The modern management of type 2 diabetes should start with prevention. Lifestyle changes based around diet and exercise have been shown over 4-5 years to reduce the progression of impaired glucose tolerance to type 2 diabetes by 52%. However, to translate trials data to a public health perspective is difficult and challenging. There is a case for the use of drugs such as the Glitazones and Metformin in the prevention of diabetes among high risk groups, along with lifestyle measures.

The prevalence of type 2 diabetes is escalating worldwide, resulting in a huge cost implication to treat the condition and its complications. Recent publications have provided new foundations for the management of people with type 2 diabetes; these are Clinical Guidelines and Evidence Review for Type 2 Diabetes: Management of Blood Glucose produced by the National Institute for Clinical Excellence, and the National Framework for Diabetes Standards and Delivery Strategy produced by the Department of Health. There is a consensus to maintain treatment targets of haemoglobin A1c level <7%, fasting

plasma glucose ≤ 6.0 mmol/l, blood pressure $< 140/80$ mmHg, total cholesterol < 4.8 mmol/l, HDL cholesterol > 1.2 mmol/l and triglycerides < 1.7 mmol/l.

The traditional first line treatment of type 2 diabetes involving diet and weight reduction and exercise are rarely sufficient in the long-term and eventually most patients require drug treatment to control their glucose levels.

Monotherapy with a single oral hypoglycaemic agent is usually effective at maintaining glycaemic control for a few years as highlighted in the UKPDS, showing haemoglobin A1c levels declined in the first year of monotherapy with either Metformin or a Sulphonylurea. About 50% of patients will require more than one pharmacological agent 3 years after diagnosis, and by 9 years after diagnosis 75% of patients will require combination therapy to maintain plasma glucose and haemoglobin A1c values at acceptable levels.

The natural history of type 2 diabetes involving an underlying insulin resistance and progressive deterioration in pancreatic beta cell function present in almost all patients with type 2 diabetes, explains the progressive loss of glycaemic control in type 2 diabetes. Insulin resistance is associated with several cardiovascular risk factors: hyperglycaemia, hypertension and dyslipidaemia are established associations. Targeting insulin resistance in the management of type 2 diabetes is of relevance.

The choice of a first-line agent should be Metformin in overweight patients and also in patients who are not overweight. If Metformin is contra-indicated or not tolerated, a Sulphonylurea should be used as a first-line agent. If glycaemic control is not achieved a second agent should be added to either Metformin or a Sulphonylurea. Depending on the first-line agent, an insulin sensitiser, Sulphonylurea or a post-prandial glucose regulator could be used. At this stage the addition of an agent that improves insulin sensitivity is of rational choice for patients with inadequate control on monotherapy, targeting the underlying insulin resistance and progressive beta cell dysfunction using combination therapy agents.

If glycaemic control is not achieved within 6 months as evidenced with haemoglobin A1c levels maintained over 7%, the next step is triple therapy with the addition of another oral hypoglycaemic agent with different mode of action or conversion to insulin. The combination of insulin treatment with Metformin is common practice and occasionally insulin is combined with Sulphonylureas.

Overwhelming evidence supports tight glycaemic control, reduces the risk of complications related to diabetes and a target haemoglobin A1c of $< 7\%$ is a goal to achieve. Initial management of type 2 diabetes with diet and lifestyle changes is rarely sufficient in the long-term and consideration should be given to pharmacotherapy at an earlier stage to attain the haemoglobin A1c level targets. Regular review of patients allows review of medication, education and regular change of therapy. Most patients

with type 2 diabetes will need combination therapy to achieve target haemoglobin A1c level as there is often progressive loss of glycaemic control due to an underlying insulin resistance and progressive pancreatic beta cell dysfunction.

However, current drugs available for the management of type 2 diabetes are not ideal, but the correct use of these drugs, initially alone and later in combination, can improve glycaemic control.

The short and long-term effects of diabetic education at preston

Binu Krishnan, Wendy Heckels, Sue Lea and A. Lakhdar
Lancashire Teaching Hospitals NHS Trust, Preston, UK.

The aim of this study was to explore the effect of a single episode of one to one diabetic and dietetic education on short and long-term glycaemic control. **Patients and Methods:** We studied 42 diabetic individuals with poor glycaemic control (Haemoglobin A1c level $> 7.5\%$) identified at diabetic annual review. 39 subjects were followed up for 1 year. All patients attended a one to one session of diabetic and dietetic education with the diabetes nurse specialist and specialist diabetes dietician, covering various aspects of diabetic and dietetic education. Patients' mean age was 52.46 ± 13.47 years (range 19-77 years). 20 were males. Body mass index mean 28.46 ± 5.73 . Haemoglobin A1c level measured at zero, 6.4 ± 2.1 and 13.4 ± 2.9 months. 24 patients had a change in therapy while medication dose was adjusted in all patients. **Results:** Mean haemoglobin A1c level dropped from $9.2 \pm 1.3\%$ at baseline to $8.0 \pm 1.5\%$ ($p < 0.05$) and to $8.3 \pm 1.2\%$ at 12 months ($p < 0.005$) in all patients. In comparison with females, male patients sustained improvement in glycaemic control up to 12 months with haemoglobin A1c level at $9.1 \pm 1.2\%$ at baseline, $7.8 \pm 1.3\%$ at 6 months and $7.8 \pm 1.8\%$ at 12 months ($p < 0.05$) comparing with female levels of $9.4 \pm 1.4\%$ at baseline, $8.3 \pm 1.7\%$ at 6 months and $9.1 \pm 2\%$ at 12 months. The pattern of haemoglobin A1c level change was not affected with alteration of treatment, race or initial haemoglobin A1c level. **Conclusions:** A single episode of one to one diabetic and dietetic education improved glycaemic control at 6 months, with a trend towards improvement at 1 year. Men responded better than women to educational strategies, suggesting a gender difference in response to educational intervention. These results reinforce the role of diabetic education as an important strategy in the management of patients with diabetes.

Mechanisms underlying contractile dysfunction in type I induced diabetic cardiomyopathy

Thomas Netticadan
Institute of Cardiovascular Sciences, St. Boniface Hospital
Research Centre, Canada

Contractile dysfunction is a feature of type I diabetic hearts. Because the sarcoplasmic reticulum (SR) plays a central role in cardiac contractility we examined the hypothesis that

abnormal SR function and its regulation contribute to contractile dysfunction in diabetic hearts. Diabetes was induced in male Sprague-Dawley rats by an injection of streptozotocin (65 mg/kg i.v.), and the animals were sacrificed 6 weeks later for assessment of cardiac performance and SR function. Cardiac performance was depressed in diabetic animals and this decline was associated with a reduction in SR Ca²⁺-uptake and -release activities. Reduced SR function was consistent with a significant decrease in the level of SR Ca²⁺-cycling proteins, such as ryanodine receptor and Ca²⁺-pump ATPase and a decrease in the ratio of protein content of Ca²⁺-pump ATPase to phospholamban. On the other hand, the calcium-calmodulin dependent protein kinase (CaMK) and cyclic AMP dependent protein kinase (PKA) phosphorylation of SR Ca²⁺-cycling proteins, the endogenous SR CaMK and PKA activities, and the endogenous SR phosphatase activities were increased in the diabetic heart. Treatment of 3-week diabetic animals with insulin partially or fully prevented the diabetes-induced changes in cardiac performance, SR Ca²⁺-uptake and -release activities, and SR protein content, whereas the diabetes-induced changes in SR CaMK- and PKA-mediated phosphorylations and activities, as well as phosphatase activities, were not significantly affected. These results suggest that the reduced content of SR Ca²⁺-cycling proteins appear to be a major defect underlying SR dysfunction and impaired contractility in the type I diabetic heart.

Management of type 2 Diabetes mellitus (NIDDM) amongst ethnic groups in the north west: is the level of help and information distributed to patients from ethnic minorities adequate to meet their individual needs?

H. Kauser

University of Central Lancashire, Department of Biological Sciences

Diabetes is one of the most common chronic diseases. There are striking differences in its frequency between racial groups. Type 2 diabetes (NIDDM) accounts for 90% of all diabetes and it is more common with increasing age, obesity and in certain ethnic groups, affecting 1 in 3 Asians over 65 years of age.

In principle their management is the same but they pose special problems of culture, religion, diet as well as communication. The aim of the study was to determine how well Type 2 diabetes is managed among ethnic groups in certain areas in the North West. The main question in research was 'Is the level of help available to the Asian diabetic adequate to meet their own individual needs and does it take into consideration cultural, religious and communicational barriers to treatment?'

30 questionnaires and 10 semi-structured interviews were conducted and completed by Asian diabetes attending diabetic clinics around certain towns in the North West. Questionnaires were available in English, Urdu/Punjabi, Hindi, Gujarati and Bengali. Interviews were conducted on one-to-one basis in English and Urdu (answers were translated into English ready for analysis).

Results from the questionnaires, showed that 40% of the participants could speak and understand English fluently. 60% could not and required an interpreter to be able to communicate with their diabetic care team. 70% of the participants were on tablets and control of diet as part of their treatment for diabetes, 20% on diet alone and 10% on insulin injections. 70% of the participants did attend diabetic clinics however 30% did not. It was also found that only 40% of the participants received information packs about diabetes, its causes, management and control in a language that they fully understood, 60% did not and had to rely on family members to educate them and translate any information they received. 40% of the participants claimed that they found the conventional diet sheets available from the diabetic health teams useful, however 60% did not find, because their normal dietary habits differed to the ones mentioned on the conventional dietary sheets. 20% of the participants were satisfied with the level of help available to the Asian diabetics, 20% were not and 60% stated that the system has room for improvement.

Results from the interviews showed that 7 of the 10 participants could not speak English fluently. 50% of the patients received information packs and diabetes, its causes and management in a language that they understood for example in Urdu. 50% did not and stated that they were unaware that teaching aids such as videos and cassettes were available from their diabetic health teams in a language they could understand. It was also found that all of the 10 participants only attended their appointments at the diabetic clinics if a member of family who could understand and speak English fluently went with them, as they themselves could not communicate with the diabetic health teams. Finally all of the 10 participants expressed that a better understanding of cultures, religions and normal Asian dietary habits is required on the behalf of diabetic health professionals in order to improve the levels of help that they receive.

To conclude, an understanding of cultural, religious and dietary practices is prerequisite for giving correct advice to the Asian patient. Appropriate teaching methods, effective educational material and reliable, sensitive and confidential interpreter service are few of the areas which still need to be carefully explored if diabetes amongst ethnic minorities is to be managed and controlled effectively.