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STUDY OF GLP-1 ASSOCIATED INSULIN RESISTANCE IN TYPE-II DIABETES PATIENTS

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ABSTRACT

Type 2 diabetes mellitus is a polygenic, genetically heterogeneous, complex disorder which is rapidly approaching epidemic proportions in the developing world. Asian countries, India in particular, are becoming highly prone to the development of type 2 diabetes. Insulin resistance is often seen in the pre-diabetic state, notably in healthy subjects genetically predisposed for type 2 diabetes. In this study, there are signs of adipose tissue distribution and adipokine production in insulin resistance associated with type 2 diabetes was studied. In conclusion that the above two mentioned adipokines, angiotensin and GLP-1, disturbances in Type-2 diabetes. There are still many more adipokines to be explored and provide exciting ventures for future research.

KEY WORDS

Type 2 diabetes, Adipokines, Angiotensin and GLP-1.

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INTRODUCTION

Globally, an estimated 382 million people have diabetes worldwide, with type 2 diabetes making up about 90% of the cases. This is equal to 8.3% of the adult's population, with equal rates in both women and men. Worldwide in 2009 and 2013 diabetes resulted in 1.5 to 5.1 million deaths per year, making it the 8th leading cause of death. Diabetes overall at least doubles the risk of death. The number of people with diabetes is expected to rise to 592 million by 2035 (Yuankai 1947). Diabetes mellitus (DM) also known as simply diabetes, is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period.[2] This high

blood sugar produces the symptoms of frequent urination, increased thirst, and increased hunger. Untreated, diabetes can cause many complications. Acute complications include diabetic ketoacidosis and nonketotic hyperosmolar coma. Serious long-term complications include heart disease, stroke, kidney failure, foot ulcers and damage to the eyes (Kitabchi, 2009). Insulin plays a critical role in balancing glucose levels in the body. Insulin can inhibit the breakdown of glycogen or the process of gluconeogenesis, it can stimulate the transport of glucose into fat and muscle cells, and it can stimulate the storage of glucose in the form of glycogen (Shoback, 2008).

The concept that insulin resistance may be the underlying cause of diabetes mellitus type 2 was first advanced by Prof. Wilhelm Falta and published in Vienna in 1931, and confirmed as contributary by Sir Harold Percival Himsworth of the University College Hospital Medical Centre in London in 1936. It is well known that insulin resistance commonly coexists with obesity. However, causal links between insulin resistance, obesity, and dietary factors are complex and controversial. Dietary fat has long been implicated as a driver of insulin resistance. Studies on animals have observed significant insulin resistance in rats after just 3 weeks on a high-fat diet (59% fat, 20% carb.) Yet another proposed mechanism involves the phenomenon known as leptin resistance. Leptin is a hormone that regulates long-term energy balance in many mammals. An important role of leptin is long-term inhibition of appetite in response to formation of body fat. This mechanism is known to be disrupted in many obese individuals. Studies show that high levels of cortisol within the bloodstream from the digestion of animal protein can contribute to the development of insulin resistance.

MATERIALS AND METHODS

Study sample

The study population consisted of all patients receiving their primary care from Private hospital in Hyderabad (2010) was selected. Study sample was selected after application of a set of inclusion and exclusion criteria to overcome inconsistencies in data recording procedures.

Inclusion criteria

All patients aged more than 18 years to less than 90 years were included in the study. All outpatients receiving their medication.

Exclusion criteria

Patients with no single visit to a clinic which provides chronic diabetic care were excluded from the study. Patients who did not have their first diabetes medication prescription from a clinic providing primary care for diabetes listed in appendix A were excluded because we could not ascertain the if the patients were newly treated or patients receiving a continuation in diabetes care.

Isolation and primary culture of mature human adipocyte

Subcutaneous adipose tissue samples were obtained from subjects undergoing elective plastic surgery. Patient consent was obtained beforehand. Tissue samples were transported in DMEM: F12 medium containing 1% Pen/Strep. Blood vessels and connective tissue were removed and tissue was minced carefully under a sterile hood and tissue pieces were added into a 50 ml falcon tube up to the 10-13 ml marking. The volume was made up to 45 ml with 4% BSA, KRP buffer containing 100 U/ml collagenase. The tubes were sealed with parafilm and placed in a shaking water bath (60-80 beats/minute) maintained at 37^{o}C for 60-90 minutes. After digestion, the material was filtered with an autoclaved nylon mesh of 2,000 um pore size to remove undigested material and connective tissue. A second filtration step was done with the filtrate using a nylon mesh of 250 μm pore size. Next, three washing steps were performed with wash buffer. The adipocytes were left in the wash buffer for a few minutes so that they could float to the top.

1ml floating adipocytes was seeded with 4 ml of DMEM: F12 containing 1% Pen/Strep. After 30 minutes, media was replaced with fresh media and cells were cultured in a humidified chamber maintained at 37^{o}C. After 16 hours, pre conditioned media was collected, made into aliquots, snap frozen and stored at -80^{o}C until further use.

Cells were grown and maintained in a humidified atmosphere at 37^{o}C and 5% CO2. Human enteroendocrine NCI-H716 cells (ATCC, CCL-251) were obtained from the Pune Tissue Culture Lab Collection. The cells were grown in suspension in RPMI 1640 medium supplemented with 10% FBS, 2 mM L-glutamine, 100 IU/ml penicillin, 100 µg/ml streptomycin, 2.5 g/l glucose, 10 mM HEPES, 1 mM Na-pyruvate. Fresh media was added to the cells twice a week. Cells were counted in a Neubauer's chamber after staining with Trypan blue (1:5 dilution). Cells were split by mixing the cells thoroughly and gently with a pipette and then taking desired volume of cell suspension and growth media in a new flask. Cells were usually split when they reached 1 million/ml.

GLP-1 secretion assay

For GLP-1 secretion experiments, NCI-H716 cells were plated at a density of 4.105 (1ml/well) on 24-well cell culture plates pre-coated with Matrigel™ basement membrane matrix (BD Biosciences) diluted (1:100) in DMEM high glucose (4g/L) medium. Medium was changed every day until the experiment. Cells were cultured until they reached 80% confluency and then growth medium was removed and the cells were washed twice with warm Hank's Balanced Salt Solution without calcium and magnesium (HBSS, Invitrogen). Compounds to test were made in PBS buffer supplemented with 1 mM CaCl2. DPP-4 inhibitor was added to the effector solutions (20 µl/ml), pH adjusted to 7.2, pre-warmed to 37^{o}C and incubated on the cells (0.5ml/well) for 1 hour at 37^{o}C in a humidified incubator at 5% CO2. At the end of 1 hour, the plate was immediately placed on ice to stop further secretion; medium was collected and centrifuged at 4^{o}C for 10 minutes at 3,000 rpm to remove cell debris. Supernatant was collected and stored at -80^{o}C until GLP-1 determination. Effectors were always tested in duplicate. Cells were homogenized with 0.1 M NaOH and protein concentration per well was measured by Bradford assay (Bio-Rad, Munich, Germany). GLP-1 (active) was assayed using an ELISA (Millipore) specific for GLP-1 (7-36) amide and GLP-1 (7- 37) amide and normalized to protein content. To study the effect of cytokines on nutrient stimulated GLP-1 secretion, cells were washed and pre-incubated with test cytokines supplemented in growth medium for defined time periods at 37^{o}C in a humidified incubator at 5% CO2. After the incubation period, media was removed and cells were incubated with effector solution for 1 hour.

RESULTS AND DISCUSSION

Isolated mature adipocytes form human subjects undergoing plastic surgery were incubated in culture medium (DMEM: F12) for 16 hours to allow fat cells to secrete adipokines into the medium. The human enter endocrine cell line, NCI-H716 was exposed to these media or DMEM: F12 (control medium) for 2 hours followed by 1 hour stimulation with glucose which is a potent stimulant of GLP-1 secretion. This finding demonstrated that adipocyte-derived factors adversely affect GLP-1 output from endocrine cells with a reduction of GLP-1 secretion in a donor BMI-dependent manner. To identify which component/s of the pre-conditioned media could be responsible for the reduced response of the NCI-H716 cells to glucose, we screened few defined adipokines which have been shown to be predictive for the development of metabolic alterations. Leptin, MCP-1, IL-6, TNF-α, Angiotensin II and RANTES were individually applied on the NCI-H716 cells for defined time periods (2-24 hours) before stimulation with 10% glucose (1 hour). Supernatants were collected for GLP-1 determination by commercially available ELISA. The screening experiments revealed that RANTES (Table No.1) and angiotensin II (Table No.2) had the highest consistent and significant effects on the reduction of GLP-1 secretion in the NCI-H716 cells following stimulation with glucose. Leptin on the other hand had no effect on GLP-1 secretion, while TNF-α (Table No.3) showed a trend to reduce GLP-1 but was not significant.

Table No.1: GLP-1 secretion of RANTES [10-8M] incubation

S.No	Control					10% glucose				
GLP-1 secretion (Pmol/gm)	0	2	6	12	24	0	2	6	12	24
	1.4±2.4	145±2.1	1.3±0.2	2.0±1.68	1.9±2.2	4.4±1.4	1.6±0.98	1.7±3.2	2.7±1.8	2.4±1.9

Table No.2: GLP-1 secretion of Angiotensin II [10-6M] incubation

S.No	Control					10% glucose				
GLP-1 secretion (Pmol/gm)	0	2	6	12	24	0	2	6	12	24
	1.7±1.4	1.5±2.4	1.6±1.2	1.9±1.98	2.1±1.2	5.0±0.4	1.4±1.98	3.0±1.2	2.9±1.8	2.8±2.9

Table No.3: GLP-1 secretion of TNF-α [10-9M] incubation

S.No	Control					10% glucose				
GLP-1 secretion (Pmol/gm)	0	2	6	12	24	0	2	6	12	24
	2±1.2	1.5±2.1	1.8±1.0	3.0±0.98	2.2±1.2	6.5±0.1	3.0±0.98	3.8±1.2	3.5±1.8	3.8±0.9

CONCLUSION

Our findings suggest that the rheogenic glucose transporter SGLT1 in the intestinal epithelium is a target of RANTES that could counteract on a feed-forward loop on glucose transport via GLP-2 while GLP-1 elicits its incretin effects. As circulating RANTES levels are increased in obese and insulin-resistant individuals, this chemokine could contribute to the reduced secretion of incretins following meal-stimulation in these subjects and antagonism of its receptors could serve as a potential target in diabetes therapy.

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