Identification of biomarkers for type 2 diabetes – Analysis of a primary prevention study among Asian Indians with impaired glucose tolerance

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For my “**AMMA**”

ஈன்ற பொழுதின் பெரிதுவக்கும் தன் மகனைச்

சான்றோன் எனக் கேட்ட தாய்

\- திருக்குறள்

Translation in English:

When mother hears him named 'fulfill'd of wisdom's lore,' Far greater joy she feels, than when her son she bore.

\- Thirukkural

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No man is an island,
Entire of itself,
Every man is a piece of the continent,
A part of the main…

- **John Donne** (1572-1631)

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Gratitude is the memory of the heart. - Jean Baptiste Massieu

Thank you!!!

STATEMENT OF ORIGINALITY

This thesis is submitted to Imperial College, London in fulfilment of the requirements of the degree of Doctor of Philosophy. This thesis represents my own original work towards this research degree and contains no material which has been previously submitted for a degree or diploma at this University or any other institution, except where due acknowledgement is made.

Ram Jagannathan

ABSTRACT

Primary prevention of type 2 diabetes (T2DM) is an important strategy for curbing its rising global burden. Though lifestyle modification has provided an effective method of preventing/delaying incidence of diabetes in high-risk individuals, it has not been widely implemented even in developed countries due to its high-cost, need for expertise and difficulties in translating the benefits of lifestyle intervention to the community at large. Hence, there is an urgent need to identify an alternate mode of delivery to transmit healthy lifestyle information to high-risk individuals. In this trial, we sought to determine whether lifestyle advice through mobile phone text messaging could reduce incident diabetes compared to standard lifestyle advice in Asian Indian men with prediabetes. The study showed for the first time that mobile phone messaging is an effective and acceptable method to deliver advice and support towards lifestyle modification to prevent T2DM in men at high risk.

The identification of novel predictors for T2DM is an arduous task. The glycaemic markers of diabetes (fasting plasma glucose, 2hr post glucose load and HbA1c) are, in fact, risk factors for microvascular complications of diabetes and it was on this basis that diagnostic cut-offs' for diabetes were arrived at. Nevertheless, elevated levels of glycaemic markers in the sub-clinical, or pre-diabetic, range are associated with increased risk of progression to diabetes. However, there is already considerable deterioration of beta cell function by the time diabetic dysglycaemia occurs. The way forward is clearly to identify biomarkers that serve as reliable predictors of progression to diabetes rather than simply reflecting accompanying levels of glycaemia. In this thesis using the database of the above mentioned trial it was aimed to identify the predictors of T2DM in Asian Indian cohort with prediabetes at baseline.

The classical risk factors studied here are: 1) increased prevalence of the hypertriglyceridemic waist phenotype, 2) a combination of HbA1c and gamma glutamyl transferase and 3) a measure of beta cell compensation (disposition index) predicted incident diabetes. Among these, the disposition index was the most powerful predictor in the cohort.

In addition to these classical risk factors mentioned above, in a small nested-case control, cross sectional study, the association of adipokines (adiponectin, leptin, interleukin-6 (IL-6), retinol-binding protein4 (RBP4)) and vitamin D3 were assessed to study the mechanistic link of novel biomarkers with diabetes. In this cohort, lower levels of baseline adiponectin, and higher IL-6 and RBP4 were associated with diabetes. Though, many of these provided a novel mechanistic pathogenic link with diabetes they did not improve prediction over and above that of glycaemic measures in identifying individuals with diabetes. However, the non-glycaemic biomarkers appear to have a role in the underlying pathogenesis of diabetes.

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ABBREVIATIONS

#

2-hr PG-2hr plasma glucose

A

ALT -Alanine Transaminase

ADA-American Diabetes Association

ANOVA-Analysis of Variance

AUC-Area under the curve

ARIC-Atherosclerosis Risk In Community study

B

BP-Blood pressure

BMI-Body mass index

BRHS-British Regional Heart Study

C

CV-Coefficient of variation

CI-Confidence interval

KORA-Co-operative Health Research in the Region of Augsburg

D

DBP-Diastolic blood pressure

DCCT-Diabetes Control and Complications Trial

DPP-Diabetes Prevention Program

DPS-Diabetes Prevention Study

E

EPIC-European Prospective Investigation into Cancer and Nutrition

F

FH-Family history

FPG-Fasting plasma glucose

FSIGT-Frequent sampling protocol

G

GGT-Gamma glutamyl transferase

H

HbA1c-Glycosylated haemoglobin

HR-Hazard ratio

Health ABC - Health, Aging, Body Composition

HDL-C-High density cholesterol

HOMA-IR-Homeostasis model assessment of insulin resistance

HTWP-Hypertriglyceridemic waist phenotype

I

IFG-Impaired Fasting Glucose

IGT-Impaired Glucose Tolerance

IDPP1-Indian Diabetes Prevention Programme-1

IDRF-India Diabetes Research Foundation

IRAS-Insulin Resistance and Atherosclerosis study

IGI -Insulinogenic index

IL-6 -Interleukin-6

IDF-International Diabetes Federation

IVGTT-Intravenous glucose tolerance test

IQR-Interquartile range

iEW-Isolated enlarged waist

iHTG-Isolated hypertriglyceridemia

K

KEGG-Kyoto Encyclopedia of Genes and Genomes

M

MISI-Matsuda's insulin sensitivity index

MESA-Multi-Ethnic Study of Atherosclerosis

N

NIN-National Institute of Nutrition

NGSP-National Glycohemoglobin Standardization Program

NAFLD-Nonalcoholic fatty liver disease

NPDR-Non-Proliferative Diabetic Retinopathy

NGT-Normal Glucose Tolerance

NWTG-Normal waist-line and triglycerides

NNT-Number Needed to Treat

NHANES-National Health and Nutrition Examination Survey-3

O

OR-Odds ratio

OGTT-Oral glucose tolerance test

P

PON1-Paraoxonase-1

PCOD-Polycystic ovarian syndrome

PROSPER-PROspective Study of Pravastatin in the Elderly at Risk trial

R

ROC-Receiver operating characteristic

RBP-4-Retinol binding protein 4

RR-Risk reduction

S

SMS-Short message service

STRING-Search Tool for the Retrieval of Interacting Genes

SBP-Systolic blood pressure

T

TC-Total cholesterol

TTM-Trans Theoretical Model

TG-Triglycerides

T2DM-Type 2 diabetes

U

UK-United Kingdom

UKIERI- United Kingdom India Education Research Initiative

USA-United States of America

V

VAT-Visceral adipose tissue

W

WC-Waist circumference

WHO-World Health Organization

1 PROLOGUE

"... Today there are 382 million people living with diabetes. Without concerted action to prevent diabetes, in less than 25 years' time there will be 592 million people living with the disease."

- Sir Michael Hirst, President, IDF.
Melbourne, 2013

1.1 "Epidemic" of type 2 diabetes

The cataclysmic increase in type 2 diabetes (T2DM) has become a major healthcare burden. According to the recent global update by the International Diabetes Federation (IDF), 382 million (8.3%) adults aged 20–79 years had diabetes in 2013; this number is expected to rise to 591.9 million by 2035[1]. For India, the IDF estimated that there were 65.1 million people with diabetes in 2013, with a projected rise of 67.4% to 109.0 million by 2035[1].

The development of T2DM is preceded by an intermediate prediabetic stage (impaired glucose tolerance (IGT) and impaired fasting glucose (IFG)) in which blood glucose is elevated when compared to normal but is below diabetic thresholds. Prediabetes is a risk state that may last for several years and increases the prognosis of developing diabetes[2]. In a meta-analysis of prospective studies published between 1979 and 2004, when compared to normoglycaemic individuals the relative risk [95%CI] for diabetes was: 5.52 [3.13-7.91] in people with isolated IGT; 7.54 [4.63-10.45] in people with isolated IFG and 12.13[4.27-20.0] in people with both IFG and IGT[3]. A recent report from the IDF estimates approximately 316 million adults (aged 20–79 years) having IGT in 2013, with a projected increase by 49.1% to 471 million by 2035[1].

1.2 Risk factors for diabetes

The identification of risk factors is essential for the successful implementation of primary prevention programmes. The risk determinants for diabetes are divided into non-modifiable and modifiable risk factors. It should be emphasized that complex diseases such as T2DM result from the interaction of genetic and environmental risk factors. The most notable risk factors for T2DM are depicted in **Figure 1-1.**

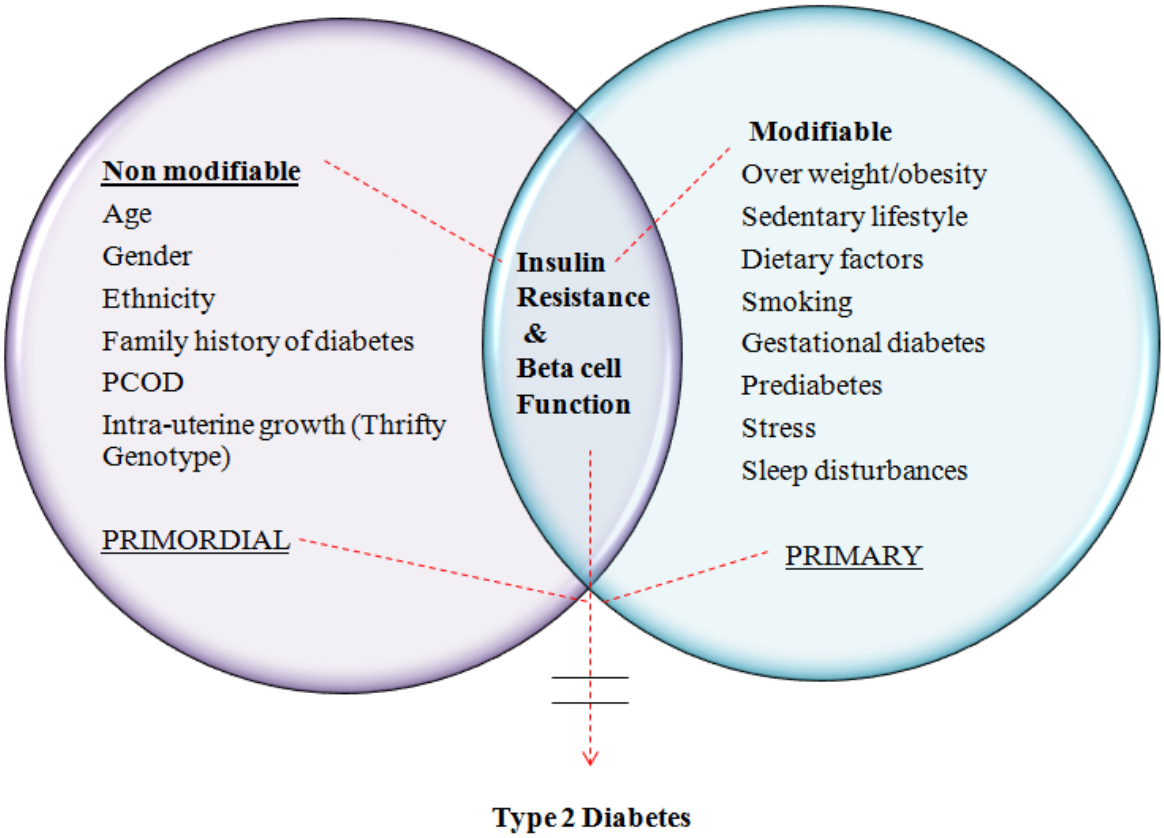


Figure 1-1: Modifiable and non-modifiable risk factors for type 2 diabetes
The major modifiable and non-modifiable risk factors for T2DM are shown. Diabetes results from an interaction of both modifiable and non-modifiable risk factors. The figure is reproduced from the review by Ramachandran et al [4]. The genetically susceptible individuals are probably predisposed to developing diabetes, either directly or through intermediate factors (e.g., weight gain and obesity), or via interactions between multiple genes as well as between genes and environmental/behavioural factors.

1.2.1 Non-modifiable risk factors

Some of the most prominent non-modifiable risk factors include: age, parental longevity, positive family history of diabetes and ethnicity, a history of gestational diabetes, polycystic ovarian syndrome and adverse intrauterine environment. T2DM aggregates in families[5]. The lifetime risk (at age 80 years) for T2DM has been calculated to be 38% if one parent had T2DM[6]. If both parents were affected, the incidence of T2DM in the offspring is estimated to approach 60% by the age of

60 years[7]. Longevity is a complex process resulting from genetic or environmental risk factors, and their interactions with families[8,9]. More than 100 genes are shown to be associated with human longevity, including those in the insulin/insulin-like growth factor-1pathway, lipoprotein metabolism (Apolipoprotein E and paraoxonase-1), and cell cycle regulators[10]. The findings from US diabetes prevention program (DPP) showed that parental longevity is inversely associated with diabetes incidence in individuals at high risk of T2DM compared with offspring's whose parents died prematurely[11]. Family studies, including those involving monozygotic or dizygotic twins, and differences in diabetes prevalence between ethnic groups, indicate that the heritability of T2DM exceeds 50%[12-16]. Ethnicity is also one of the main contributory factors for the development of diabetes. Though, all ethnic groups are at risk of T2DM, certain non-white Caucasian groups, such as Indo-Asians and Native Americans, appear to have a particularly strong genetic predisposition[17].

1.2.2 Modifiable risk factors

Some of the major modifiable environmental risk factors include: sedentary behaviour, nutritional over-indulgence, overweight or obesity, central adiposity, rapid urbanization, smoking, increased psychological stress and sleep irregularities[18-22]. The scientific evidence, relative risk and mechanism of action of important modifiable risk factors are summarized in **Table 1-1**.

Table 2-1: Association between modifiable and non-modifiable risk factors for type 2 diabetes and disease development

Risk factors	Scientific evidence	Hazard ratio [95%CI]	Proposed mechanism
Overweight / obesity	Meta-analysis: 18 prospective cohort studies [23]	Obesity: 7.28 [6.47- 8.28] Overweight: 2.92 [2.57-3.32]	Increased insulin resistance and increased pancreatic β- cell compensation and increased ectopic fat accumulation.
Smoking	Meta-analysis of 25 prospective studies in multi-ethnic cohort [24-26]	1.61 [1.43-1.80]	Smoking has been identified as a possible cause of insulin resistance, a precursor for diabetes. Smoking has also been shown to cause deterioration in glucose metabolism, which may lead to the onset of T2DM. There is also some evidence that suggests smoking increases diabetes risk through a BMI-independent mechanism.
Sleeping	Quantity and quality of sleep and diabetes: 10 studies in multi-ethnic populations [27]	Short duration: 1.28 [1.03–1.60] Long duration: 1.48 [1.13–1.96] Difficulty in maintaining sleep: 1.84 [1.39 –2.43] Difficulty in initiating sleep: 1.57 [1.25–1.97]	Reciprocal changes in circulating levels of leptin and ghrelin. These in turn would increase appetite and caloric intake, reduce energy expenditure and facilitate the development of obesity and impaired glycaemic control, increasing cardiovascular risk. Increased cortisol secretion, acute-inflammatory biomarkers and altered growth hormone metabolism have also been implicated.

Table 1-1: Association between modifiable and non-modifiable risk factors for type 2 diabetes and disease development (Continued)

Risk factors	Scientific evidence	Hazard ratio [95%CI]	Proposed mechanism
Lack of physical activity	Sedentary habits and television watching [28,29]	TV watching (2h/day increment): 1.14 [1.05-1.23]	Alter energy expenditure by displacing time spent on physical activities, TV viewing is associated with unhealthy eating (eg, higher intake of fried foods, processed meat, and sugar-sweetened beverages and lower intake of fruits, vegetables, and whole grains)
Diet	Increased white rice consumption [30] Meta-analysis of 4 studies (2 Asians and 2 Western)	1.55 [1.20-2.01] A dose-response analysis showed that each serving per day of white rice consumption was associated with an 11% increase in risk of diabetes in the overall population	White rice consumption leads to an increased glycaemic load which results in increased insulin resistance and pancreatic beta cell exhaustion.
	Increased consumption of red meat: Meta-analysis of 10 studies in multi-ethnic populations [31].	1.51 [1.25, 1.83]	Mediated through the effects of heme-iron derived from red meats; Iron is the pro-oxidant and increase oxidative stress thereby causing damage to tissues.
	Sugar sweetened beverages: 8 prospective studies in multi-ethnic populations [32]	1.26 [1.12–1.41]	Increased sugar sweetened beverages leads to increased weight gain and worsens cardiovascular risk profile.
	Fruit and vegetable intake and diabetes: Meta-analysis of 6 multi-ethnic observational studies [33].	0.86 [0.77-0.97]	The plausible mechanisms responsible for the beneficial effects of fruits and vegetables on diabetes risk are likely to be diverse. Besides their contribution to low energy intake, high fiber content, and low glycemic load, fruits and vegetables are also rich in antioxidants, vitamins, magnesium, potassium, plant proteins, and other individual phytochemicals, which could be beneficial in reducing risk of T2DM.

	Coffee, tea consumption and diabetes: Meta-analysis of 18 studies in multi-ethnic cohort [34].	0.93 [0.91-0.95]	The biological mechanism responsible for the inverse relationship between coffee and tea consumption and T2DM is unclear. The protective effect was independent of magnesium, potassium, caffeine or blood pressure effects. Coffee is rich in the antioxidant chlorogenic acid and tea contains flavonols and flavones as antioxidants which reduce oxidative stress. Therefore, it improves insulin sensitivity and beta cell function.

1.3 Pathophysiology

The development of T2DM involves both environmental and genetic components as described in the previous sections[35]. The recent epidemic of the disease cannot be attributed primarily by genetic influences because these factors do not change in a short duration. Therefore, a marked worsening of unhealthy environmental risk factors such as decreased energy expenditure, increased calorie intake and stress may be the main causative factor for the increasing prevalence of diabetes and its associated complications. This concept is broadly referred to as "gene × environment interaction", in which the "gene" is usually one or more DNA variants and the "environment" can be any nongenetic factor that impacts risk[36]. Furthermore, the in-utero environment, maternal under nutrition (prior to and during the pregnancy) and lower birth weight (defined as the proportion of newborns weighing less than 2,500 grams (the measurement taken within the first hours of life, before significant postnatal weight loss has occurred)) could result in epigenetic and gene-expression changes that affect the risk of development of obesity and T2DM for the offspring. The incidence of low birth weight is higher in the South East Asian regions compared with developed nations. Furthermore, Indian babies are the smallest in the world. Approximately one-third of the child born is of low birth weight [37]. This has been attributed to the "thrifty phenotype" in which a low birth weight due to poor intrauterine growth followed by a rapid childhood weight gain due to nutritional transition and physical inactivity promotes development of obesity and associated metabolic complications[38,39]. Pooled estimates of 14 studies involving a total of 132,180 individuals showed that low birth weight (<2,500 g), as compared with a birth weight of 2,500 g or more, was associated with increased risk of T2DM (odds ratio (OR) 1.32 [95% CI: 1.06, 1.64])[40].

According to the current understanding of the pathophysiology of diabetes, both insulin resistance and first phase insulin secretion are the important determinants for the development of diabetes. Individuals with impaired glucose regulation were maximally/near maximally insulin resistant and have lost almost 80% of the β-cell function. Therefore, decompensation of β-cell function relative to the prevailing insulin sensitivity is important for the development of clinical diabetes because without a relative deficiency of insulin production, hyperglycaemia would not occur[41,42]. In addition to the liver, muscle and the β-cell (triumvirate), the fat cell (accelerated lipolysis), gastrointestinal tract (incretin deficiency/resistance), α-cell (hyperglucagonemia), kidney (increased glucose reabsorption), and brain (insulin resistance, appetite regulation) all play important roles in the development of impaired glucose regulation (ominous octet) [43,44].

1.3.1 Insulin resistance

Insulin resistance is a conspicuous feature of the prediabetic state. Insulin resistance in apparently healthy individuals differs from more extreme endocrine system abnormalities in that there is not a complete loss of the action of insulin, but rather a relative defect in the insulin response in target organs, especially liver[45].

The obese, insulin resistant individual has disrupted pulsality of insulin secretion, higher plasma levels of free fatty acids and increased inflammatory cytokines[46,47]. However, the relationship between dysglycaemia and obesity is complex and may be modified by ethnicity[7]. For a given range of body mass index (BMI), age-matched, apparently healthy Asian Indians were more insulin-resistant, had reduced insulin clearance rate, greater hyperinsulinaemia and poorer lipid profile compared with white populations[7] and are thus at increased risk of developing metabolic syndrome[48]. Several multi-ethnic studies have

found that the strength of association of diabetes with anthropometric measures might differ between Asians and Caucasians[49].

1.3.2 Pancreatic Beta cell dysfunction

Pancreatic β-cell dysfunction is a critical determinant for T2DM and is compounded by insulin resistance[50,51]. Glucose is a major regulator of transcription and translation in pancreatic β-cells and a critical regulator of β- cell function[52]. A decrease in β-cell mass of >60% has been reported in T2DM [53]. Multiple factors, including genetic predisposition[54], gluco-lipo toxicity[55], impaired pulsatile insulin secretion [45] and pancreatic steatosis[56], exacerbate insulin resistance, which ultimately leads to the development of β-cell dysfunction [43,57,58]. Finally, inefficient proinsulin processing to insulin[59] and a reduction in the release of islet amyloid polypeptide, known also as amylin[60], have been observed in established T2DM. In the majority of ethnic groups decreased 2-hr OGTT insulin levels and impaired first phase insulin secretion (insulinogenic index) predict conversion of IGT to T2DM[61-63]. In addition, the first-degree relatives of patients with T2DM exhibit impaired pulsatile insulin secretion with a complete loss of regular oscillatory capacity[64,65]. Therefore, novel interventions designed to preserve β-cell function and / or increase β- cell mass are of paramount importance to prevent/delay conversion of IGT to T2DM.

1.3.3 Insulin resistance and beta cell measures in the prediction of T2DM

From the aforementioned chapters it is clear that decreased insulin action and inadequate insulin secretion are central features in the pathogenesis of T2DM. Hence, the ability to accurately measure insulin action and/or early phase insulin secretion is of substantial importance to identify high-risk individuals. In order to measure insulin sensitivity and β-cell function clinicians have used euglycaemic-hyperinsulinemic and hyperglycaemic

clamp techniques [66] and intravenous glucose administration according to a frequent sampling protocol (FSIVGT). However, these techniques are time consuming, invasive, labour intensive and not feasible in large epidemiological settings. Consequently, a number of simple (surrogate) indices derived from transformations or weighted combinations of insulin or insulin and glucose concentrations in the fasted state and at various time points during the OGTT have been proposed as alternate simple tools to measure insulin secretion and action[67-71]. In fact, several prospective and cross-sectional epidemiological studies demonstrate that high insulin resistance and low insulin secretion often precede the onset of diabetes in different race/ethnic groups with varying states of glucose tolerance, family history of diabetes, or obesity [61,72-74]. Recently, prospective studies have shown that the product of insulin secretion and insulin sensitivity (disposition index), can be used to identify high-risk individuals according to the inability of their β-cell to compensate for the compounding insulin resistance[75-79]; importantly, disposition index appears to decline well before glucose levels rise[80]. Thus, a low disposition index is an early marker of inadequate β-cell function[81,82].

1.4 Diagnosis of type 2 diabetes

The present diagnostic cut points for diabetes (fasting plasma glucose (FPG) of 7.0 mmol/l and 2-hr post oral glucose load (OGTT) plasma glucose (2-hr PG) of 11.1 mmol/l) are derived based on glycaemic levels above which a substantially increased risk of diabetes-associated microvascular complications is apparent, particularly diabetic retinopathy [83,84] (**Table 1-2)**. These criteria exploit the observation that there appears to be a clear glycaemic cut-off that separates persons at high and low risk of diabetic retinopathy[85,86]. In 2009, an International Expert Committee that included

representatives of the American Diabetes Association (ADA), the IDF, and the European Association for the Study of Diabetes (EASD) recommended measurement of glycated haemoglobin, HbA1c, (the 'A1C test') to diagnose diabetes, with a threshold of ≥6.5%, and the ADA adopted this criterion in 2010 [87]. The A1C test has several advantages over FPG and OGTT, including greater convenience (since fasting is not required), greater preanalytical stability, and less day-to-day perturbations during periods of stress and illness. Recently, a data-pooling analysis of nine studies from five countries with 44,623 participants aged 20 –79 years with gradable retinal photographs from DETECT-2 collaborators suggests that the current FPG level for diagnosis of diabetes should be lowered to 6.5 mmol/l and that an A1C of 6.5% (47.5 mmol/mol) is a suitable alternative diagnostic criterion[88].

Based on the current recommendations from the World Health Organization (WHO), pre-diabetic states may also be distinguished as: IFG with a range of FPG of 6.1–6.9 mmol/L (in the absence of IGT) and IGT defined as 2-hr PG of 7.8–11.0 mmol/L based on the 75g glucose load OGTT, or a combination of both. The ADA also recommends using HbA1c 5.7-6.4% (38.8-46.4 mmol/mol) for diagnosis of pre-diabetes (using the term 'category of increased risk for diabetes') [84].

Table 2-2: Diagnostic criteria for type 2 diabetes

Diabetes test	**Diagnosis of diabetes**	**Prediabetes / IGT / IFG**
HbA1c (%; mmol/mol) * Using a method certified by NGSP and standardized to the DCCT assay[89]; or	≥6.5% (47.5)	5.7-6.4 (38.8-46.4)
Fasting plasma glucose (mmol/l) * (Fasting is defined as no caloric intake for at least 8 hrs) or	≥7.0	6.1-6.9**
2-hr plasma glucose (mmol/l) **(OGTT)** * The test should be performed as described by the WHO, using a glucose load containing equivalent of 75 gms of glucose dissolved in water; or	≥11.1	7.8-11.0
Random blood glucose (mmol/l) In a patient with classic symptoms of hyperglycaemia	≥11.1	7.8

*: In the absence of unequivocal hyperglycaemia, criteria 1–3 should be confirmed by repeat testing. NGSP: National Glycohemoglobin Standardization Program; DCCT: Diabetes Control and Complications trial; OGTT: oral glucose tolerance; IGT: impaired glucose tolerance; IFG: impaired fasting glucose.
Table adopted from [84];
**: IFG diagnostic criteria for ADA: 5.6-6.9 mmol/l.

The existing glucose-based diagnostic tests, FPG and the 2-hr PG derived from the OGTT, though they are rapid and inexpensive, have well-known performance limitations [90,91]. The observed variability in the course of prediabetes is mostly because of inherent variability in glucose tolerance (particularly as evaluated by the OGTT). Although, determination of HbA1c was a better and alternate marker for chronic hyperglycaemia, it must be stressed that HbA1c measurement alone is not a sufficiently reliable tool for recognizing particularly the early stages of T2DM or prediabetes. For instance, analyses of the U.S. National Health and Nutrition Examination Survey (NHANES-3) data from 2005-2006 indicate that, assuming universal screening of the undiagnosed, the A1C test cut point of ≥6.5% (47.5 mmol/mol) identifies one-third fewer cases of undiagnosed diabetes than a fasting plasma glucose cut point of ≥7.0 mmol/L[92]. Similar observations were also noted in other studies[93-95]. In essence, HbA1c is an alternative marker for microvascular risk, but it a weak marker to identify undiagnosed diabetes[96].

1.5 Economic burden of treating diabetes

T2DM poses a huge fiscal burden on national health care systems [97]. The IDF estimated that the global annual health expenditure due to diabetes in 2013 has been more than $578 billion[1]. This health care expenditure may continue to increase if diabetes continues to be undiagnosed within an appropriate time. Globally, 174.8 million of all diabetes cases in adults are estimated to be undiagnosed[98]. India, accounts for nearly 31.9 million cases of these cases. A study based on the American population showed non-diagnosing diabetes in patients resulted in expenditure of an additional $18 billion in healthcare costs per year [99]. Thus, early identification and prevention of diabetes at initial stages is paramount for public health priority to prevent diabetes-associated

complications and disability. There is also a huge disparity in diabetic healthcare spending between age groups, gender, regions and countries. For instance, the average cost of treating diabetes in the USA is $9800 / person; whereas it is $84.4 for a patient living in India (i.e. less that 1% of the expenditure in the USA) [1]. Therefore, the challenge is immense, particularly in developing countries like India, which are undergoing rapid socioeconomic transition, directly linked with the adoption of a "*Western lifestyle*", mainly in terms of diet and physical activity habits.

1.6 Need for the prevention of diabetes

The enormous human and financial costs that accompany T2DM, and the challenge of treating it effectively once it has developed, make it an appropriate target for prevention[100]. Identification of individuals at high-risk of developing T2DM, especially at the prediabetic stage is itself the pre-eminent strategy to prevent diabetes and its associated complications. It is to be noted that prediabetes is not a benign condition. In addition to the risk of progression to diabetes, the presence of prediabetic condition can itself increase risk for vascular complications that traditionally are attributed to diabetes. For instance, data from the DPP research group showed that 7.9% of individuals with IGT and 12.6% with newly diagnosed diabetes were affected with non-proliferative diabetic retinopathy[101]. Several prospective and cross-sectional studies have demonstrated an increased risk of neuropathy and cardiovascular risk in individuals with prediabetes [102]. Hyperglycaemia is a well-established risk factor for cardiovascular disease[103]. A meta-analysis of 34 prospective studies showed a modest association between increased cardiovascular disease risk and prediabetes [104]. Thus, prevention of

diabetes by modalities that prevent or delay the conversion of prediabetes to T2DM is the need of the hour to reduce the global burden of diabetes.

Primary prevention is defined as the prevention of a disease by controlling modifiable risk factors through population prevention programs[105]. Primary prevention strategies are broadly classified as: a) downstream programs which target individuals having the highest risk of the disease (e.g, prediabetes or obesity); b) midstream approaches which target populations or communities found to be at increased risk of diabetes (eg, Pima Indians, Asian Indians); and c) upstream programs which target the whole population, which include public policy and environmental interventions planned to increase the support for maintaining a healthy lifestyle. The high risk / downstream program approach is the appropriate strategy for primary prevention of diabetes. For identification of high-risk individuals, non-invasive multivariable risk scores derived from age, gender, weight, family history of T2DM and physical activity [106-110] have been found to be simple and economical as the cost of screening by blood tests in large numbers can be avoided.

1.7 Prevention of diabetes

Table 1-3 shows the details of the major diabetes prevention studies that used lifestyle modification as an intervention. The Finnish Diabetes Prevention study (DPS) [111] and American DPP [112] found that the risk of T2DM could be reduced by 58% in individuals with IGT over a period of 3 years compared with control. Similar results were also observed in Swedish [113], Chinese [114] and Japanese [115] populations. The results from Indian Diabetes Prevention Programme-1 (IDPP-1), a community-based, randomized, controlled study in 531 participants with persistent IGT (421 men and 110 women) showed that diabetes is preventable in high-risk, comparatively lean, Asian Indians. The study aims were to find out whether the progression to diabetes could be postponed by interventions using lifestyle modification by repeated personal contacts, metformin, or a combination of both in Asian Indians with IGT who were younger, leaner, and more insulin resistant than the Caucasian population. The results at the end of 30 months follow-up showed that all three modalities of intervention were equally effective (relative risk reduction approximately 28.5%). There was no additional benefit by combining lifestyle modification and metformin[116].

Table 2-3: Landmark primary prevention studies using lifestyle modification in primary prevention of diabetes

Study *	Study population characteristics	No of patients by treatment group	Duration (Years)	Lifestyle goals	Cumulative incidence (%)	RRR (%)
Da Qing Study (1997)[114]	Chinese Mean BMI: 26 Mean Age: 45	Control: 133 Diet: 130 Exercise: 141 Diet + Exercise: 126	Mean, 6	Weight loss and maintenance of healthy diet and / or exercise	Control: 67.7; Diet: 43.8 Exercise: 41.1 Diet + exercise: 46	Diet: 31 Exercise: 46 Diet + exercise: 42.0
Diabetes Prevention study (2001)[111]	Finnish, White Mean BMI: 31.1 Mean Age: 55.0	Control: 257 Intervention: 265	Mean 3.2	Reducing weight, total intake of fat, and intake of saturated fat and increasing intake of fibre and physical activity.	Control: 23 Intervention: 11.0	58.0
DPP Research Group (2002)[112]	Multi ethnic: 3234 Mean BMI: 34.0 Mean Age: 50.6	Control: 1082 Intervention: 1079	Median, 2.8	7% weight loss + 150 min exercise per week	Control: 11 Intervention: 4.8 (Effective in Asian American)	54.0
Indian Diabetes Prevention Programme-1; (2006) [116]	Asian Indian Mean BMI: 25.8 Mean Age: 45.9	Control: 136 Intervention: 133	Median, 2.6	Weight maintenance by restricting refined carbohydrates and fat + 30 min exercise	Control: 55 Intervention: 39.3	28.5
Japanese Prevention Programme. (2005) ** [115]	Japanese Men Mean BMI: 23.5 Mean Age: 51.5	Control: 102 Intervention: 356	Mean, 4.0	Reduction in BMI to ≤22 kg/m2 by 30–40 min exercise per day	Control: 9.3 Intervention: 3.0	67.4

BMI: Body mass index; RRR: relative risk reduction; *:All study population had impaired glucose tolerance at the baseline of the study; **: in this study, the oral glucose tolerance test used 100g glucose and modified criteria for recruitment.

1.8 Prediction of diabetes

Identification of the prediabetic stage (IGT and IFG) has been used to identify individuals with high risk for T2DM. Indeed, all the major landmark primary prevention studies that have evaluated intervention strategies for preventing T2DM have recruited individuals with IGT[111,112,114-116] for assessing the benefits of intervention strategies. However, in large, prospective, epidemiological studies, only ~ 30-40% of the individuals with IGT/IFG develop overt T2DM eventually[117-119]. Furthermore, ~40% of individuals who develop T2DM have NGT at baseline [117]. This limits the use of IGT/IFG as the sole means to identify individuals at high risk for T2DM.

Dysregulation of many biological pathways leads to the development and progression of T2DM[120]. The measurement of glucose alone cannot reflect the complexity of metabolic disorders arising from a combination of excessive adipose tissue[121], peripheral insulin resistance[120], dysfunctional pancreatic β- cells[122], increased free fatty acids[123,124], impaired incretin effects[125], abnormal glucagon secretion[126] and obesity-induced inflammation[127,128]. Hence, it is imperative to identify simple, inexpensive and accurate predictive markers to identify high risk individuals.

1.9 Structure and overview of thesis:

In the context of diabetes prevention it is important to identify improved strategies to disseminate the principles of lifestyle modification to the individuals at high risk of developing T2DM. Importantly, it is imperative to identify simple, routinely measurable – "predictors" and novel biochemical markers for better identification of the progressive pathogenesis of diabetes.

The main objectives of this thesis are:

- To determine whether lifestyle advice through mobile phone text messaging could reduce incident diabetes in Asian Indian men with prediabetes compared with standard lifestyle advice.
- To identify the association of glycaemic measures, and routine measures such as gamma-glutamyl transferase, enlarged waist, hypertriglyceridemia and surrogate insulin measures with incident diabetes.
- To unravel the association of mechanistic biomarkers such as adiponectin, leptin, retinol binding protein-4, interleukin-6 and Vitamin D3 with incident diabetes.

This thesis is structured around five chapters to achieve the above research objectives. Each of these chapters is described separately, as a standalone chapter with relevant background information, statistical methods and in-depth discussion. Below is the brief description of the content of all following chapters:

- **Chapter-2:** Study design and methods
- **Chapter-3:** Primary and secondary outcomes of the study and mechanism of action of intervention.
- **Chapter-4:** Association of classical risk factors (glycaemic markers, liver enzymes, hypertriglyceridaemic waist and surrogate insulin measures) with incident diabetes was studied.
- **Chapter-5:** In a small nested, case-control study, the association of novel risk factors (adipokines and 25-hydroxy vitamin D_3) with incident diabetes was studied.
- **Chapter-6:** overall summary and conclusions.

2 METHODOLOGY

2.1 Study design

This was a prospective, parallel-group, randomised controlled trial at 10 sites in southeast India between Aug 10, 2009, and Nov 30, 2012. Total duration of the study was 2 years with 6 monthly reviews. The trial synopsis is shown in **Table 2-1**. In total, individuals with impaired glucose tolerance (IGT) on two repeated occasions at baseline were randomly allocated either into: arm:1 - control arm, which received standard care advice at baseline only; and arm:2- an intervention arm that received automated, repeated motivational messages about healthy lifestyle habits in addition to the standard lifestyle advice (ClinicalTrial.Gov no: NCT00819455).

2.2 Objectives

The primary objective was to assess the effect of educational and motivational text messaging (short message service, SMS) by mobile phone in reducing incident cases of diabetes among prediabetic individuals. Secondary objectives were to study the improvements in: (1) body mass index (BMI) (kg/m^2); (2) waist circumference (cm); (3) systolic and diastolic blood pressure (mmHg); (4) lipid profile - total and high density lipoprotein cholesterol (mmol/l); (5) serum triglycerides (mmol/l); (6) dietary energy intake by 24- hr food recall diary (kcals); and (7) physical activity score by questionnaire in the intervention group compared with control group. Acceptability of SMS by the participants was assessed during the follow-up visits in the intervention arm only.

Table 2-1: Trial synopsis

Title:	The Role of Information Technology in the Primary Prevention of Type 2 Diabetes (NCT00819455)
Principal Investigators:	Prof. Dr. A. Ramachandran (IDRF) Prof. Desmond G Johnston (Imperial college, London)
Funding source:	India Diabetes Research Foundation (IDRF) & Dr. A. Ramachandran's Diabetes Hospitals, India
Collaborators:	Imperial College, Faculty of Medicine, London
Sponsors:	United Kingdom India Education Research Initiative (UKIERI), British Council, UK
Trial design:	Prospective, Parallel design, randomized, controlled trial
Population:	Asian Indian men; middle class, industrial workers
Main inclusion criteria:	Non-diabetic; Age: 35 – 55 years; BMI $\geq$ 23.0 Kg/m^2; positive family history; ability to read messages; sedentary lifestyle.
Main exclusion criteria:	Known diabetes; physical conditions impeding exercise such as arthritis, knee surgery, physically challenged etc., known serious illness (cancer, heart problems); mental illness; illiterate.
Study arm:	Standard advice: Received personalized education and motivation on healthy lifestyle principles and written information on diet and physical activity. Intervention: Received personalized lifestyle advice at baseline. Also, the intervention group received educational and motivational messages through tailored text messages at frequent intervals for two years.
Planned sample size:	Standard advice: 257; Intervention: 257
Study Duration:	Two years (6 months interim visits)
Primary objectives:	The effect of educational and motivational text messaging (short message service, SMS) by mobile phone in reducing incident diabetes among prediabetic individuals.
Secondary objectives:	Improvement in body mass index; waist circumference; systolic and diastolic blood pressure; total and HDL cholesterol; triglycerides; dietary energy intake and physical activity by questionnaire; overall compliance with lifestyle advice.
Endpoint:	Conversion to diabetes

2.3 My responsibility

Field visit:

- Site identification and contacting the occupational health officers and administrative officials.
- Involved in the field visit throughout the study.

Message construction and questionnaire development:

- Assist psychologist with the questionnaire development (thoughtful inputs to improve / simplify the transtheoretical model inventory and SMS acceptability questionnaire(contribution- my role: 30%; psychologist:40%; dieticians and physicians: 30%)).
- Assist psychologist in text messages development (simplifying the messages, provided study materials for adding the messages, construction of diabetes awareness messages (contribution- my role: 30%; psychologist:40%; dieticians and physicians: 30%)).
- Involved in the development of web-based software algorithm for sending the messages (created proto-type along with Intel corp, Bangalore, India (contribution-my role: 30%; software personnel: 70%).
- Conceptualized, implemented and helped in the development of computerized diet inventory tool (contribution-my role: 50%; software personnel: 30%; dietician: 20%; complied with the national institute of nutrition, India)
- Grouping of the participants according to transtheoretical model stages (already scored by the psychologist) in the website and delivery of the text messages to the intervention group participants (contribution-my role: 60%; psychologist : 40%.

Database management:

- Created spreadsheet template specific for this study to capture all the data efficiently with appropriate data entry validations (automatic coding, data validation and conditional formatting of the data).
- Data entry (screening, randomization and follow-up visits data); in charge of complete database (to date).

Biochemistry and data analyses:

- Blood sample collection, separation, aliquoting and storage.
- Biochemical estimations specific for this trial.
- Conceptualized and created automated laboratory reports (given to the participants) specific for this trial.
- Setting up the laboratory (research) and estimation of biomarkers.

- Statistical data analysis (planning and performance of main paper results; conceptualization and statistical analysis plan for subsequent ancillary post hoc data analysis)

2.4 Study population

In order to recruit participants with IGT, 10 private and public sector organisations with a large number of employees (>500) in Chennai, Tamil Nadu and Visakhapatnam, Andhra Pradesh, India were identified. Because > 96% of the employees were men, we included only men in the trial. Initially, we planned to recruit along family members into the trial however this could not be implemented owing to the differences in the randomization allocation procedures (cluster randomization, with permuted blocks of random size and number of participants in the family [one vs more than one]) involved in recruitment. Furthermore, the intervention principles employed in this trial was based on the healthy lifestyle changes in an individual rather than that of family based intervention techniques. Participants' jobs were self-classed as unskilled, skilled, clerical or executive.

2.5 Eligibility criteria and screening

At baseline, eligibility criteria were: no diabetes (self-reported) or major illness, such as cancer, chronic liver or kidney disease; no disorders with cognitive impairment, severe depression or mental imbalance; no physical disability that would prevent regular physical activity; no recruitment in another trial; age 35–55 years; ownership of a mobile phone and ability to read and understand mobile phone messages in English; a positive family history of type 2 diabetes (T2DM); and a BMI of 23 kg/m² or more.

After screening for eligibility, capillary blood glucose was measured with a glucometer (Accu-Check Sensor, Roche Diagnostics, Mannheim, Germany) at the participants' workplace 2-hr after consuming 75 g oral glucose.

2.6 Confirmatory oral glucose tolerance test / Recruitment

During the confirmatory oral glucose tolerance test (OGTT), we collected venous blood samples at fasting, at 30 min and 2-hr after 75 g glucose consumption; study staff checked to make certain that all of the solution was consumed. Blood was collected into an EDTA and serum tube, was centrifuged for 20 minutes at 2000 RPM at room temperature, and frozen to -20°C until the sample was assayed for biochemical, insulin and for the estimations of biomarkers. Two-hour plasma glucose (2-hr PG) was estimated using a glucometer (Accu-Check Sensor, Roche Diagnostics, Mannheim, Germany) at the participant's place of work using a drop of venous blood collected during the OGTT. The correlation between the glucometer (capillary blood glucose measurement) and venous plasma glucose values were tested in 100 blood samples and it was found to be highly correlated ($r=0.91$, $P<0.0001$ with coefficient of variation (CV) 4.9%) between the readings.

2.7 Ethical statement

All participants provided written informed consent prior to baseline assessments (see appendix-1). The informed consent process involved communicating information about the study aims, procedures, expectations, confidentiality, risks, and benefits associated with participating in the research study. The risks to participants in this study were minimal and included lab assessments that could cause slight discomfort (e.g., blood draw) as well as questions about behaviours and feelings that could cause psychological distress. Benefits included receiving results from study assessments. The study protocol was approved by the Ethics Review Committee of the India Diabetes Research Foundation, Chennai, India. All the study data were stored (with restricted access) in locked file cabinets.

2.8 Randomisation and masking

A central investigator not involved in analysis of trial data used a computer-generated randomisation sequence (Matlab randperm version 6) based on Marsaglia's algorithm[129] to randomly allocate patients (1:1) to individually-tailored mobile phone messaging or to a control group that received standard lifestyle modification advice at baseline only.

Laboratory personnel and principal and co-investigators were masked to the participants' group allocation until the end of the study. Field staff and participants were, by necessity, not masked.

2.9 Lifestyle advice

Although the study was conducted in two different states of the country, the participants' lifestyles were similar with regard to diet and physical activity practices owing to the proximity of the geographical location. During recruitment, all participants were educated on the causes, effects, symptoms, treatment, and management of diabetes, they were also educated on the possibility of preventing or delaying the onset of T2DM. The participants' queries regarding diet and physical activity were answered and beliefs regarding diabetes were also clarified.

The participants were randomly allocated into control (standard lifestyle advice only at baseline) and intervention (individually tailored TTM based text messages for two years) group.

The standard lifestyle advice followed a holistic approach, taking the following key healthy lifestyle factors into consideration, as explained by the nutritionist: healthy and unhealthy food products, foods to be avoided or limited, the benefits of vegetables and fruits; the ill effects of sweets, sugars and carbonated drinks were also explained. The amount of oil to be consumed by a family based on their family size was also explained.

Their daily nutrient intake was assessed by 24h recall method. Based on the self-reported diet practices, all subjects were given an individualized written diet chart to achieve the desired goals. These goals were to reduce portion size (total calories) and, to avoid simple sugars and refined carbohydrates, reduce total fat intake, restrict use of saturated fat, include more fibre rich food-(e.g., whole grains, legumes, vegetables, and fruits). Subjects who had been following these healthy diet practices were encouraged to continue the same and changes were recommended if there was a need to change their diet habit. The details of the diet prescription are given in **table 2.2**.

Participants were educated on the benefits of physical activity. The physical activity advice was intended to be simple and easy to follow and was tailored to the participant's present activity level. In sedentary subjects, the recommendations were to initiate or enhance aerobic exercise like walking, cycling, and jogging in sedentary subjects. Brisk walk for minimum 30 minutes per day (or equivalent) on all days, or at least five days a week; walk 3 – 4 km in 30 min at least 5 days a week; cycle 6 -7 Km in 30 min at least 5 days a week. If the participant's occupation involved strenuous work or if participants were already engaged in walking or cycling for more than 30 min a day the advice given was to continue the same. Sedentary participants were advised to initiate physical activity, participants with light activity were advised to increase physical activity and participants were asked to maintain the level in order to achieve and maintain ideal bodyweight and glycemic improvement. The prescribed physical activity and diet recommendations are shown in **table 2.2** and were similar to those used in a previous trial in India[116].

Participants, both in the control and intervention groups, were advised to achieve healthy lifestyle goals at baseline. The goals comprised of: a) decreased consumption of carbohydrates; b) decreased consumption of oil; c) decreased portion size; d) decrease in BMI of at least 1 unit (1 kg/m^2) from baseline; and e) to sustain good physical activity. At

the end of the follow-up, the number of goals scored were calculated according to participants' success in achieving the lifestyle principles (success score between 0 and 5) using standard procedures. For those who developed diabetes, the values at the time of diagnosis were considered as the final data.

Individuals were given a grade for achieving each goal of the intervention at the end of the study (with "0" indicating goal not achieved and "1" indicating the goal achieved), and a success score was computed as the sum of these grades. The possible association between incidence of diabetes and lifestyle goals achieved was calculated using logistic regression analyses. The success score of ≥ 3 goals was compared with < 3 goals (reference category) after adjusting for baseline values of age, BMI and 2-hr PG. Since both the groups received standard lifestyle advice at baseline, the intervention and control groups were pooled together for this analysis.

After prescribing lifestyle changes, we reassessed all participants clinically and biochemically every 6 months from baseline. No additional lifestyle information or advice was routinely given by personal contact after the baseline visit in either the control or the intervention groups, except in response to specific queries from participants.

Table 2-2: Dietary advice and physical activity recommendations to the participants

Dietary advice:

The dietary recommendations were individualized to balance food intake and physical activity and to maintain appropriate body weight. The advice includes:

a) Avoid simple sugars and refined carbohydrates
b) Reduce total fat intake (not to exceed 20 g/day)
c) Restrict use of saturated fat
d) Include more fibre-rich food – whole grains, legumes, vegetables and fruits.

Dietary adherence:

- Poor: not following the advice for more than 5 days a week (non-adherent)
- Fair: occasional deviation, following advice for 2-4 days a week (adherent)
- Good: strictly following diet advice for more than 5 days a week (adherent)

Physical activity recommendation:

a) To enhance aerobic exercise like walking, cycling and jogging in sedentary individuals
b) Brisk walk for a minimum of 30 min/day (or equivalent), as a realistic goal which has proven efficacy
c) Walking 3 – 4 Km in half an hour at least five days a week
d) Cycling 6 – 7 Km in half an hour
e) If occupation involves strenuous work , no specific advice

Physical activity adherence

- Poor: less than 150 min per week (non-adherent)
- Fair: 150-250 min per week (adherent)
- Good: more than 250 min per week or if occupation involved strenuous work (adherent)

Ramachandran A, Snehalatha C, Ram J, et al. Lancet Diabetes Endocrinol 2013 [130].

2.10 Text messaging (intervention)

Both control and SMS groups received lifestyle recommendations at baseline as described above. Only the latter subsequently received educational and motivating SMS at frequent intervals.

The SMS content at any time was based on the transtheoretical model (TTM) stages of change, based on the following principles. The TTM model explains how people vary in terms of motivational readiness to quit unhealthy behaviour and move through specific stages for behaviour change. The TTM consists of 5 stages of change (precontemplation, contemplation, preparation, action and maintenance) developed by Prochaska and DiClemente [131,132]. Each stage is unique in its characteristics: **Precontemplation**: no awareness of the benefits of healthy lifestyle, hence no change; **contemplation**: aware but has not taken any realistic steps toward the goal, intend to change in the next six month; **preparation:** working out strategies to meet the desired goal; **action**: practicing healthy lifestyle for less than six months duration; **maintenance**: following healthy lifestyle more than six months and working to prevent relapse to the previous stage.

According to this model, people use both cognition and behavioural strategies and techniques to progress through these 5 stages which are described as process of change. The first 3 stages involve changes at the cognitive level, including:

- Create awareness and increase awareness
- Awareness raising of the ill effects associated with unhealthy lifestyle
- Impact of unhealthy lifestyle on his or her family, friends and at the community level
- Benefits of healthy lifestyle
- Increase awareness of opportunities for physical activity and healthy diet practices.
- The fourth and fifth stage involves changes at the behavioural level including:

- To adhere to healthy lifestyle even when stressed out or tired or when there are opportunities to slip down
- To associate with people who aspire to achieve similar goals
- To reward or reinforce themselves when achieving desired goals
- To stay more active and follow healthy diet
- To avoid cues for unhealthy lifestyle and to leave reminders for healthy lifestyle

An essential aspect of this work was to define a subject's TTM stage with respect to physical activity and dietary habits. In order to assess the effect of tailored text messages in transitions in TTM stages in the intervention group and to compare them with the control group, the researcher constructed a project-specific TTM inventory. This comprises a questionnaire of 20 items, 10 items each to determine the participant's TTM stage in relation to dietary habits and physical activity practices (appendix) .

A two stage pilot study was conducted in the development of this tool. The first stage of the pilot study was conducted among 10 members of the study team. Modifications were made based on the feedback and suggestions from the team members about the items. The second stage pilot study was conducted among 40 non-diabetic volunteers and further modifications were made based on the response from these non-diabetic volunteers. The present version of the questionnaire was approved to be used in this study after 'face validation' (comprising an expert assessment tool, viewed as covering the concept it proposed to measure) by two health psychologists.

It is well-documented that TTM model is a widely applied behavioural theory in health research, however there is no sufficient data to show the usage of the Prochaska's originally developed TTM questionnaire in healthy behavioural lifestyle research. Hence the researcher developed the questionnaire specifically to meet the study objective.

According to Prochaska's stages of change model, duration of lifestyle practices 6 months is the criterion to decide the subject's TTM stage on a particular behaviour. The questionnaire was developed taking duration of diet and physical activity practices into consideration but although duration of an individual's habits is the major criteria for stage assessment, additional questions were posed to ascertain their actual diet and physical activity practices. These additional questions are called lie scales. Lie scales are the set of items or questions included in a psychological evaluation to ascertain whether or not the respondent has been truthful in answering the other items in the questionnaire. The responses to the questions are yes or no format. Question numbers 1, 3, 6 and 9 assesses the participants TTM stage, and the other 6 questions are lie scales. Similar questionnaire was constructed to assess the TTM diet stages of change. The questionnaire and scoring key are given in the appendices.

2.10.1 TTM based message construction

The SMS content at any time was based on the transtheoretical model (TTM) of behavioural change[131,132]. A TTM stage-based SMS delivery manager algorithm was created in a partnership with the Intel Corporation, India (C# programming language). The SMS messages were delivered by a commercial service provider (Unicel technologies, India). They contained <160 characters and there were 60-80 messages for each TTM stage, with simple words and easy to understand. The SMS content was on the causes, effects and prevention of diabetes and other associated lifestyle diseases. SMS messages on benefits of healthy lifestyle, pros and cons of changing and not changing the behaviour; cues to initiate or start physical activity and overcome unhealthy dietary habits were delivered. Strategies to avoid or overcome relapse to the previous unhealthy behaviour and to sustain healthy lifestyle were also included. In general, the objective of the SMS was to create awareness, assist, guide and support the individual to change unhealthy lifestyle and

motivate to move across stages by providing various cues and strategies to follow and remain motivated throughout the life. It was constructed based on the fact that participants would not normally receive the same message in a 6 month period (based on their receiving 2-4 messages per week).

2.10.2 Concepts in the development of text messages

As mentioned earlier, TTM is the underlying behavioural theory in this intervention. It consists of 4 core constructs; the "stages of change", "process of change", "decisional balance", and "self-efficacy". The stages of change (precontemplation, contemplation, preparation, action and maintenance stage) is the main construct of this model which explains the steps involved in progressing to the desired behaviour. Although, stages of change is the major construct of this model which demonstrates the benefit of SMS through change in subject's progression and regression between stages, the process of change explains how people change their behaviour. The detailed description of the processes of change is shown in table 2.3. Briefly, processes of change include ten cognitive (i.e. involving thinking) and behavioural (i.e. involving action) strategies that can help subjects' to make changes and maintain them. The cognitive processes include; consciousness raising, dramatic relief, environmental re-evaluation, self-re-evaluation, social liberation. The behavioural processes of change are counter conditioning, helping relationships, reinforcement management, self-liberation, and stimulus control. These strategies state that, different strategies (process) are most effective and relevant at different stages of change when people move from one stage to the other. For example, counter-conditioning and stimulus control can really help people in the action and maintenance stages, but these processes are not helpful for someone who is in the first three stages.

Table 2-3: Transtheoretical model - the processes for each stage of change

Stages	Process of change	Explanation
Precontemplation to Contemplation	Consciousness raising	Increased awareness of causes, consequences, and cues to overcome the problem; increasing information about self and the unhealthy behaviour
	Dramatic relief	Experiencing and releasing feelings about the possible consequences of the behaviour; using feelings to help motivate change.
	Environmental re-evaluation	Cognitive and affective assessments of how the presence or a absence of the behaviour affects one's social environment; becoming aware that one can serve as a positive or negative role model to others
	Social liberation	Recognizing changes in the environment or social changes that influence personal change
Contemplation to Preparation	Self re-evaluation	Cognitive and affective assessments of the subject's self-image with and without behaviour in order to change how one thinks about oneself in relation to the behaviour
Preparation to Action	Self-liberation	Recognizing choices related to available actions and making a commitment to change a behaviour
Action to Maintenance	Reinforcement management	Applying consequences in the form of rewards to oneself for making changes
	Helping relationships	Seeking and accepting support from others in the form of caring, trust, acceptance, and openness
	Counter conditioning	Learning new and healthier alternative behaviours to substitute for the unhealthy behaviour
	Stimulus control	Avoiding or removing environmental cues for unhealthy behaviour and adding cues for healthier alternatives

2.10.3 Validation of the SMS

The investigator compiled the messages as a comprehensive list of short statements (the SMS) which incorporate five cognitive and five behavioural strategies of processes of changes. The messages covered both physical activity and dietary dimensions. The SMS were validated by the following validation process method. The SMS were analyzed by 5 expert psychologists, specialized in health psychology, psychological training assessments and psychometrics. In the first step, the experts were asked to rate each message for its loading as a cognitive or behavioural component. The ratings were collected based on concurrence for each item, the higher the concurrence the more valid the message. In the next step, the experts were asked to classify the SMS in any one of the ten process strategies based on the principle that the processes differ when people move from one stage to the other. For example, cognitive processes of change (consciousness raising, dramatic relief, environmental re-evaluation, social liberation and self-re-evaluation) are relevant for the first three stages and behavioural processes (reinforcement management, helping relationship, counter conditioning, stimulus control and self-liberation) are relevant for the action and maintenance stage. Messages were written taking the characteristics of the above variables into consideration.

The details of the message construction along with the examples of the messages were given below:

2.10.3.1 *Pre-contemplation:*

Pre-contemplation is the stage in which an individual has no intent to change behaviour in the near future, usually defined as the next six months. Individuals at this stage may not be informed or may lack information about the consequences of their behaviour, or they have attempted to change their behaviour and failed and, therefore, are demoralized in their ability to change their behaviour. These people are often characterized as resistant or

unmotivated and tend to avoid information, discussion, or thought with regard to the targeted health behaviour[132,133].

Example messages – physical activity

- Walk for 60 minutes a day
- Regular walking keeps you healthy and fit
- Exercise helps to reduce the risk of heart disease
- Physical activity helps to maintain normal blood sugar and blood pressure
- Lifestyle modification enhances quality of life
- Active life makes you live longer
- Physical activity is good for your heart.

Example messages – Diet

- Eat healthy, be healthy and be happy
- Too much of anything is bad
- Regular eating pattern helps to maintain normal blood sugar
- Moderate use of starchy food, oil, sweets and sugars are advisable
- Do not skip any meal
- Fruits have antioxidants, vitamins, minerals which are essential for daily life
- Maintain good health

2.10.3.2 *Contemplation*

Contemplation is the stage where individuals openly state their intent to change within the next six months. The individuals have increased awareness of the benefits of changing but are still considering the cost involved in changing the behaviour. These people are seriously undecided to change and are stuck at this stage for a longer period of time. They are also known as contemplators or procrastinators and are often not ready for traditional action-oriented programs[132,133].

Example messages – physical activity

- Moderate physical activity keeps you healthy
- Feeling bored, go for a walk
- Desk-bound job? Take short walk to relax your body and mind
- Actively apply your body muscles to some tasks (running, swimming, sports, stair climbing and stretching) for good health

- Ideal young man becomes unhappy old man, be active always
- A good exercise can keep you mind fresh

Example messages – Diet

- Avoid snacks while watching T.V. you may over eat
- Take timely food and avoid over eating
- Intake of fruits, best natural protection against high BP and high cholesterol and there by prevent heart diseases
- Neither fast nor feast, have a balanced diet
- Avoid sweets and sugars in any forms as it increase your blood sugar

2.10.3.3 *Preparation*

Preparation is the stage in which the person intends to take steps to change, usually occurring within the next six months. These individuals have attempted some important action in the past and most often have a plan of action, for example attending health education classes and talking to the counsellor. These are the people who are best suited for action-oriented programs. However, the individuals have not met the criteria for effective action and can be considered as at the early stirrings of the action stage[132,133].

Example messages – physical activity

- Use stairs instead of lift
- Park you vehicle at far end of the parking area and walk to your destination
- Walk to the place of worship or market, which is close by
- Include walking / cycling as part of the daily routine like going to market, place of worship or even to your office
- Choose a form of exercise that suits your interest

Example messages – Diet

- Starchy and oily foods need to be consumed in moderate
- Increase fibre rich foods, fruits and vegetables and whole cereals
- Do not skip any meal, you may over eat at the following meal
- Make your plate colourful by adding lot of vegetables
- Eat one fruit daily as a snack
- Avoid over eating at any meal
- Fruits are delicious and nutritious; include them as part of your diet

2.10.3.4 *Action*

Action stage refers to when people made overt modifications in their lifestyles within the past six months. Individuals must meet the criteria agreed by professionals to reduce the risk of a disease. Action is defined as the most explicit behavioural transformation and needs considerable commitment of time and energy. For example, a successful change of addictive behaviour means achieving a specific criterion such as abstinence[132,133].

Example messages – physical activity

- All you need is 30-45 minutes of moderate physical activity on most days of the week
- Hope it had been a healthy week
- Physical activity helps in proper digestion
- Be relaxed, anger is never without a reason, but seldom with a good one
- A good exercise can keep your mind fresh
- The key to relaxing your body is to focus on the physical sensation of relaxation

Example messages – Diet

- Take six split servings instead of 3 big servings of food
- Have a healthy meals always
- Use sugar free in moderate quantities
- Regular intake of 8 to 10 nuts a day is good for the hear
- Avoid sweets and sugars in any form to keep normal blood glucose
- Take fruits as a whole and not as a juice

2.10.3.5 *Maintenance*

Maintenance is the stage in which individuals work to avoid relapse and are most often less tempted to deteriorate as they increasingly become confident and able to continue their changes. Conventionally, maintenance was viewed as a static stage, whereas it is actually a continuation and not merely an absence of change. Thus, the characteristics of this stage are stabilizing behaviour change and avoiding relapse[132,133].

Example messages – physical activity

- Were there many missed walks this month
- Are you stressed out? Stress increases blood sugar, go for a walk and relax
- Physical activity helps to control or reduce weight
- Mental agitation can affect you. Hope enables as to endure the hardships of physical and mental life.

Example messages – Diet

- Skipping breakfast will make you overeat at lunch
- Avoid fried and salty foods
- Healthy diet is essential, relish your food
- Drink water when you feel like drinking beverages
- Junk food is no way healthy, avoid it

The assumption was that by motivating participants on healthy lifestyle principles through SMS they will move from a pre-action stage to an action stage. The timing and frequency of messages were tailored to the participants' preference assessed at the 6 monthly visits. Although, SMS in the participants' own vernacular is ideal, this could not be implemented due to the fact that large number of basic mobile phones (personal digital assistant (PDA)) were not compatible to multiple language systems (to South Indian languages such as Tamil and Telugu). The contents of the messages were based on: a. To emphasise the benefits achieved by healthy lifestyle habits; b. Simple reminders to reinforce participants to practice and adhere to the principles of lifestyle modification; c. Information about the consequences of progression to diabetes; and d. Methods to sustain healthy lifestyle habits.

2.11 Measurements:

2.11.1 Anthropometric measurements

Supine Blood pressure (BP) was measured (mean of two readings) at each visit using a sphygmomanometer. Height and weight were measured to the nearest 0.1 cm and 0.5 kg, respectively (BMI was calculated). Waist circumference was measured midway between the lower rib margin and the iliac crest using a steel measuring tape to the nearest 0.1cm.

Body fat percentage was measured using bioimpedence method (OMRON, Karadia Scan, Japan).

2.11.2 Clinical measurements

Clinical assessments specific for the trial is given in the **table 2.4**. We have used COBAS Intergra 400 plus automated system for estimating the routine clinical measures and plasma insulin was estimated in Elecsys® analyzer.

Table 2-4: Clinical assessments performed during the trial

Clinical test	Principle	Coefficient of variation	Detection range
Glucose	Hexokinase method	<3.0	0.11-25 mmol/l
HbA1c	Turbidimetric inhibition immuno assay; Tina-quant® HbA1c	<1.5	Hb: 4 - 40 g/dL; HbA1c: 0.3-2.6 g/dl
Triglycerides	GPO-PAP	<2.0	0.05-11.3 mmol/l
Total cholesterol	CHOD-PAP	<2.0	0.08-20.7 mmol/l
HDL cholesterol	HDL plus-3rd generation	<3.0	0.08-3.10 mmol/l
gamma-glutamyl transferase	GGT-2	<2.0	3-1200 U/L
Alanine transaminase	ALT IFCC with pyridoxal phosphate activation	<4.0	4-600 U/L
Insulin	electrochemiluminescence immunoassay	<3	1.39–6945 pmol/L

2.12 Questionnaires

Physical activity and dietary intake were assessed by questionnaires completed by field workers at baseline and during the 6-monthly reviews. Assessments were done by seven field staff trained in undertaking glucose tolerance tests, questionnaire administration and biometric measurements. Training was provided by central training staff with use of continuous training methods previously used in our diabetes projects in India[116]. Each person had a designated role to minimise inter-individual error.

2.12.1 Physical activity

Physical activity was quantified on a score of 7–70.The activity questionnaire was based on that used previously in south-Asian Indians in a UK epidemiological survey [134,135], which we used in our previous study of diabetes prevention in India, but was slightly modified for the Indian environment [116]. The copy of the physical activity questionnaire and the scoring schema (published material; appendix-2) are provided in the accompanying appendix. Researcher did not play a role in developing this questionnaire as it was already validated and has been used extensively in other observational [134,135] and primary prevention programmes[116,136] conducted by our group.

2.12.2 Habitual dietary energy intake

At each visit we assessed dietary intake by 24-hr recall. The energy intake from individual food constituents was calculated with a dietary analysis program using the National Institute of Nutrition guidelines for India [137]. The details of the 24hr recall questionnaire and the development of diet analyses software are given in the appendix-3. Information about adherence to recommendations for dietary intake and physical activity was recorded at the 6-monthly reviews. Adherence was self-reported, on the basis of weekly patterns, and was scored as poor, moderate, or good.

2.12.3 Text messaging acceptability

A project-specific SMS acceptability questionnaire comprising of 8 items was developed by the researcher (psychologist). A similar SMS acceptability questionnaire was used in a clinic-based study in which the utility of SMS was tested to reinforce Asian Indian diabetes patients visiting their hospital clinic to adhere to prescription recommendations[138]. The study showed that frequent communication via SMS was acceptable to diabetic patients and it helped to improve their health outcomes. The questionnaire used in the present study was a modified version with questions to suit the study's specific needs. This modified questionnaire was intended to help measure the acceptability, frequency, difficulty in understanding, advantage, preferred time and problems due to the SMS. One question invites participant's suggestions to improve. The response to the questions was "Yes or No" (scoring: yes: 1; no: 0), and each correct response was given a score of 1. The SMS acceptability questionnaire was used at each follow up to assess the acceptance of the SMS received by the intervention group. The assessment also helped the researcher to make changes in the frequency, timing and type of SMS (diet or physical activity) delivered to the subjects. A copy of the questionnaire is included in appendix-4. A total score of 6 was the most acceptable and 0 the least.

2.13 Surrogate insulin measures caluculations

HOMA-IR[67] was calculated using the formula: (fasting insulin (mU/l X fasting glucose (mmol/l)) / 22.5); since, it is a measure of insulin resistance the inverse of HOMA-IR (i.e., 1/HOMA-IR) was used to express insulin sensitivity. Matsuda's insulin sensitivity index (ISI) was calculated by the formula: (10^4/ square root of (fasting glucose * insulin) X (mean OGTT glucose * mean OGTT insulin)), with mean glucose and insulin calculated from values at fasting, 30 and 2-hr of the OGTT test[139]. The insulinogenic index (IGI) was calculated as the ratio of the change in insulin to the change in glucose from 0 to 30

minutes following the oral glucose load ($\Delta I_{0-30}/ \Delta G_{0-30}$)[61]. Total area under the curve (AUC) for insulin and for glucose were calculated using the trapezoidal rule, and a ratio of the two was calculated ($AUC_{ins/glu}$)[140].

2.14 Definition of variables

- Diabetes was diagnosed on the basis of an annual oral glucose tolerance test (OGTT) or a semi-annual 2-hr post glucose load test, according to the World Health Organization criteria [2]: plasma glucose of a value of 126 mg /dl (7.0 mmol/l) or higher in the fasting state or 11.1 mmol/l or higher two hours after a 75-g oral glucose load. During interim visits, if the values were ≥11.1 mmol/l by 2-hr PG test, an OGTT was performed within a week to re-confirm the diagnosis of diabetes.
- BMI between 23.0 and 24.9 kg/m^2 was considered as overweight, and BMI ≥ 25.0 kg/m^2 was defined as obese [141].
- Central obesity was indicated by waist circumference of ≥ 90 cm [6].

  Individuals with a history of hypertension and newly diagnosed cases with blood pressure readings ≥ 130/85 mmHg (on two repeated occasions) were categorized as hypertensive.
- Hypertriglyceridemia: Fasting serum triglycerides ≥ 1.7 mmol/l[6].

Figure 2-1 shows the study timeline and progress of the study. The total duration of the study was 40 months (screening period: 16 months). The schedule procedures are given in **Table 2-4.**

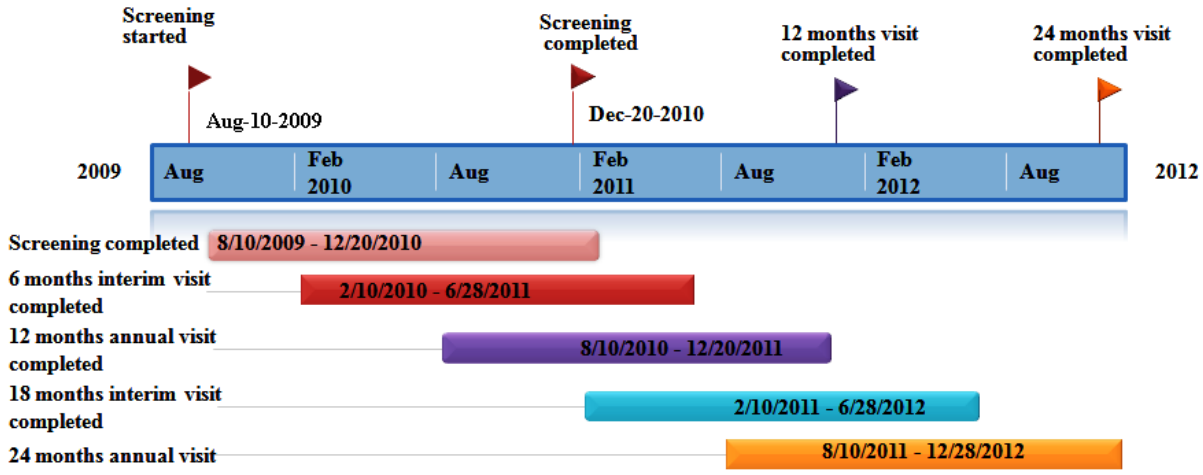


Figure 2-1: Trial - Study timeline

Table 2-5: Schedule procedures

	Baseline	6 months	12 months	18 months	24 months
Informed consent	X				
Questionnaires [a]					
Demographic	X				
Clinical History	X	X	X	X	X
Habitual dietary pattern	X	X	X	X	X
Physical activity	X	X	X	X	X
General Health Awareness	X	X	X	X	X
Quality of life	X	X	X	X	X
Transtheoretical model assessment	X	X	X	X	X
Text messaging acceptability [b]		X	X	X	X
Anthropometric [a]					
Height (Cm)	X	X	X	X	X
Weight (Kg)	X	X	X	X	X
Waist circumference (Cm)	X	X	X	X	X
Body fat (%)	X	X	X	X	X
Blood pressure (mmHg)	X	X	X	X	X
Clinical measurements [a]					
3 sampling OGTT	X		X		X
2-hr post glucose load		X		X	
HbA1c	X		X		X
Lipid profile	X		X		X
Insulin estimations	X				X
Gamma-glutamyl transferase	X				X
Alanine transaminase	X				X

a: for individuals who progressed to diabetes (confirmation using 3 sampling OGTT), the value at that time point considered as a final values; for these individuals complete clinical measurements were carried out. b: only for intervention group.

3 TRIAL RESULTS

3.1 mHealth – critical review

Developing countries—both low- and middle-income—often suffer from shortfalls in medical information, access to healthcare, treatment quality and affordability, and behavioural norms. Most of these disparities stem from gaps in resources, particularly financing, infrastructural, and the availability of skilled health workers. There is a clear need for innovative, home grown solutions that use technology to leapfrog these impediments. The proliferation of mobile technology in developing countries may offer this kind of opportunity. It has been predicted that by 2017 there will be ‘‘more mobile phones than people’’ on the planet [142], and according to the current estimates, three-quarters of the world’s population have access to a mobile phone[143]. Thus, mobile phone-based interventions may offer an alternative method for delivery of educational advice and motivation to achieve lifestyle modification[131,144]. Early in its development, in 2003, m-health was defined as wireless telemedicine involving the application of mobile telecommunications and multimedia technologies and their integration with mobile healthcare delivery systems[145]. Especially, text messaging (short message service, SMS) by mobile phone is an alternative means of delivery of educational advice and motivation to achieve healthy behaviour changes[131,138,144,146] . The major advantages of mHealth include:

- Temporal synchronisation - many people carry their mobile phone with them wherever they go. As mobile technologies can be transported wherever one goes, interventions are convenient and easy to access.

- Real-time (synchronous) communication - allows interventions to be accessed or delivered within the relevant context, i.e., the intervention can be delivered and accessed at any time and wherever it is needed.

- The crux of mHealth is the exchange of information . It is easy, low cost and highly translational to a community at large.

Table 3-1 shows the details of the important studies which employs text messaging system in various behavioural and reminder systems such as smoking cessation, improvement of physical activity, diabetes management and improvement in patient adherence.

Table 3-1: Trials using SMS as an intervention strategy in behavioural change

Study	Research design and participants	Sample size	Duration	Tailoring / frequency of messages	Intervention effects
Smoking cessation					
Obermayer (2004)[147]	Design: pre-post pilot study Setting: Colleges from the Washington DC	46	6 weeks	Yes / daily	At 6-week follow-up, 43% had made at least one 24-hour attempt to quit. 22% were quit--based on a 7-day prevalence criterion.
Rodger (2005)[148]	Design: RCT / Single blind Setting: New Zealand public	1705 Control: 853 SMS: 852	26 weeks	Yes / daily	More participants reported not smoking in the intervention group (28%) compared to the control group (13%) at 6 weeks ($p<0.0001$).
Free (2011)[149]	Design: RCT / Single blind Setting: United Kingdom	5800 Control: 2885 SMS: 2915	6 months	Yes / daily	Biochemically verified continuous abstinence at 6 months was significantly increased in the txt2stop group (10·7% txt2stop vs 4·9% control, relative risk (HR: 2·20, 95% CI 1·80-2·68; $p<0·0001$).

Study	Research design and participants	Sample size	Duration	Tailoring / frequency of messages	Intervention effects
Physical activity					
Hurling (2007)[150]	Design: RCT / no blind Setting: Bedfordshire, United Kingdom	77 Control: 47 SMS: 30	9 weeks	Yes / daily	Participants with access to a fully automated behaviour change system engaged in, on average, 2 h 18 min more physical activity per week than control.
Kirsty (2009)[151]	Design: RCT / no blind Setting: New Zealand	78 Control: 40 SMS: 38	12 weeks	No / daily	Text messaging and pedometers as motivational tools did not increase physical activity.
Adherence to medication					
Lester (2010)[152]	Design: RCT Setting: HIV-AIDS patients in Kenya (Antiretroviral therapy)	538 Control: 265 SMS: 273	1 year	No / weekly	The number needed to treat (NNT) to achieve greater than 95% adherence was nine (95% CI 5.0-29.5) and the NNT to achieve viral load suppression was 11 (5.8-227.3)

Study	Research design and participants	Sample size	Duration	Tailoring / frequency of messages	Intervention effects
Downer SR (2006)[153]	Design: Cohort study with historical control. Setting: Royal Children's Hospital, Melbourne, Victoria. (Patient reminders)	45110 Control: 22452 Intervention: 22658	1 year	Frequent appointment reminders	Failure-to-attend rate was significantly reduced in the SMS reminders group (9.8% vs. 19.5%; P < 0.001).
Vervloet (2012)[154]	Design: RCT Setting: Netherlands (diabetes- adherence to oral hypoglycaemic agents)	104 Control: 48 SMS: 56	6 month	No / daily	SMS reminders improves adherence of type 2 diabetes patients (50% vs. 39% within a 1-h window (p=0.003) up to 81% vs. 70% within a 4-h window (p=0.007)).

Though, lots of pilot, short term studies demonstrated a feasibility and applicability of SMS technology in behavioural intervention, strong supportive data are lacking, however, such that a recent World Bank Report, showed that despite more than 500 mHealth pilot studies conducted so far [155], we know almost nothing about the likely uptake, best strategies for engagement, efficacy, or effectiveness of these initiatives [156,157]. Currently, mHealth interventions lack a foundation of basic evidence, let alone a foundation that would permit evidence-based scale up [156]. There is even less evidence for mobile phone messaging in disease prevention [158] despite its potential as an aid in smoking cessation having been established [149].

3.2 Rationale

Primary prevention of type 2 diabetes (T2DM) is an important strategy in mitigating its rising global burden. As explained in **Chapter-1**, Diabetes Prevention Programmes (DPP's) conducted in different parts of the world in various ethnic and racial groups have demonstrated that by controlling the adverse effects of environmental risk factors through lifestyle modification, it is possible to reduce risk of T2DM by 30-60% [159]. Though these studies provide "proof-of-principle" for preventative strategies, their findings have not been widely implemented even in developed countries due to high-costs, need for expertise and difficulties in translating the benefits of lifestyle intervention to the community at large. Hence, there is a need to identify simple and inexpensive methods to educate and motivate people at high risk for diabetes. In this trial, we sought to determine whether mobile phone text messaging could reduce incident diabetes compared to standard lifestyle advice in people with prediabetes in India, the country with one of the largest populations of people with diabetes and expanding mobile phone accessibility [160]. This method of delivery, if successful, is potentially scalable as mobile phones are widely used

even in low and middle income countries and their ownership is largely unaffected by socioeconomic status.

3.3 Study design and methods:

Described in detail in **Chapter-2**. Please refer section 2.2.

3.4 Statistical analyses:

With the assumption of a 30% cumulative incidence of T2DM over 2 years (in our previous study in India [116], which had similar eligibility criteria, the incidence was 55% over 3 years), 214 participants per group were needed for a 40% reduction in progression to T2DM to be detected at 5% significance with 80% power. In a meta-analysis of behavioural modification studies [159] the mean reduction was 50% [159] and we assumed that mobile phone messaging would be less effective than personal contact. We aimed to recruit 514 participants (257 in each group) to allow for 20% dropout rate during follow-up.

The analysis was to be by intention to treat. Two-sided t-tests were used to analyze the differences between the groups at base line. χ^2 test was used to analyze the differences between the categorical measures. For participants who developed T2DM, we regarded the values at the time of diagnosis as final. Estimated cumulative incidence of T2DM was calculated using unadjusted Cox regression[161,162] analysis to compute the hazard ratio (HR) and survival curve for the intervention versus the control groups. The HR indicates the relative likelihood of *the pre-specified outcome* in treated vs. Control individuals at any given point in time. The mean survival time is estimated as the area under the survival curve in the interval 0 to t_{max}[163]. Absolute risk reduction (ARR), also called the risk difference, was calculated as the difference between the control event rate (CER) and experimental event rate (EER). The ninety five percent confidence interval (95%CI) for

the ARR was calculated using the formula: ARR +/-1.96 X SQRT ([CER X (1-CER)/ no. of control participants + EER X (1-EER)/ no. of experimental patients]) [164]. The number needed to treat and 95% CI to prevent one case of T2DM as the inverse of the absolute risk reduction (RR) and its 95% CI. The greater the risk, the more likely to gain from intervention [164].

To assess the effects of the intervention on secondary outcomes (and ancillary analysis variables) we used fixed effect, mixed-linear regression modelling [165,166] with maximum likelihood parameter estimation for continuous variables. The MIXED procedure fits models more general than those of the general linear model (GLM) procedure and it encompasses all models in the variance components (VARCOMP) procedure. The linear mixed-effects models (MIXED) procedure in SPSS enables us to fit linear mixed-effects models to data sampled from normal distributions. Therefore, the variables which were non-normally distributed such as triglycerides and physical activity score were natural log transformed before analysis. Intervention or control group was a fixed effect in this model. Differences in the estimated marginal means between the groups with 95% CIs with P values were shown.

We analysed categorical outcomes (adherence to diet and physical activity) with a generalised estimating equation-based logistic regression analysis [167,168], with adjustment for baseline values and time. The corresponding odds ratios (and associated 95% CIs) with P values were shown.

The change in BMI, diet adherence and physical activity were computed by subtracting final follow-up from the baseline values. For diabetic cases the values at the time of diagnosis were considered as the final values. To evaluate predictors of incident diabetes independent of intervention, Cox regression analysis was used. Prior to modelling, the

proportional hazards assumption was tested and confirmed by examining interactions between follow-up time and each predictor ($p > 0.05$ for all interactions). The variables which showed significant univariate associations with diabetes were entered for multivariable modelling. The variables included for this exploratory analysis were: age, family history of diabetes, baseline 2-hr plasma glucose (2-hr PG), HOMA-IR and insulinogenic index, and baseline and change in BMI, dietary energy intake and physical activity. Study group was not included for this analysis, since the components of lifestyle interventions (i.e., dietary and physical activity habits) were already included in the model as separate variables. All analyses were performed using SPSS software (IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp).

3.5 Results

3.5.1 Initial screening

Figure-3.1 shows the trial profile. Among the 9160 non-diabetic individuals (men: women 8801:359) invited, 9079 (men: women 8741:338) participated and were tested using the 2hr post 75g oral glucose load capillary blood glucose method. Of the 8741 individuals screened, 4692 (53.7%) had normal glucose tolerance (NGT), 2744 (31.4%) had IGT and 1305 (14.9%) had undiagnosed diabetes. Those with previously undiagnosed diabetes were advised to re-confirm the diagnosis as early as possible elsewhere with a repeat oral glucose tolerance (OGTT) and HbA1c to rule out the laboratory error.

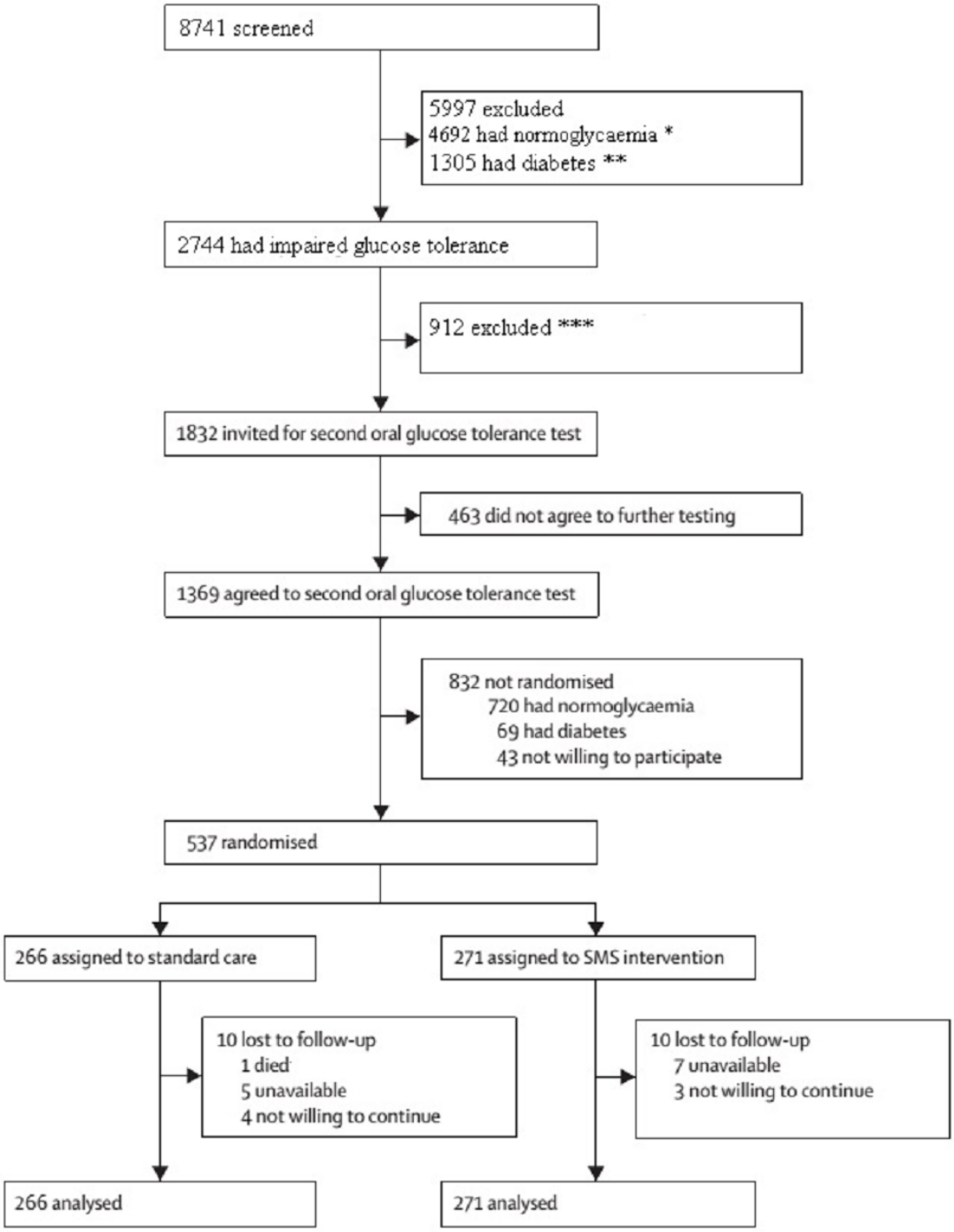


Figure 3-1: Flowchart showing screening, recruitment and follow-up details of the participants.
The diagnosis of diabetes is based on WHO recommendations: *: 2hr PG value <7.7 mmol/l; **: 2-hr PG ≥ 11.1 mmol/l; ***: in order to increase the yield during confirmatory OGTT and reduce the cost of testing, participants with 2-hr PG <8.9 mmol/l were excluded for the confirmatory testing;
Ramachandran A, Snehalatha C, Ram J, et al. Lancet Diabetes Endocrinol 2013 [130].,

3.5.2 Characteristics of the individuals in relation to the screening glycaemic results

Table 3-1 shows the characteristics of the individuals who underwent the screening in relation to their glycaemic findings. Mean age was 45.7 ± 5.0 years; positive family history of T2DM was present in 32.0%. Diabetic individuals were older, had higher BMI, waist circumference and blood pressure ($p<0.05$) as compared with normal glucose tolerance (NGT) and IGT individuals ($p<0.05$). Positive family history of diabetes, and blood pressure showed progressive increase from NGT, through IGT to T2DM ($p<0.0001$ for all).

Table 3-2: Results of screening OGTT

Characteristics	Total (n=8741)	NGT (n=4692)	IGT (n=2744)	Diabetes (n=1305)
Occupation [a] n (%) Unskilled Skilled Executive/ Clerical/ Professional	 318 (3.9) 6297 (77.3) 1533 (18.8)	 202 (4.6) 3291 (75.4) 872 (20.0)	 79 (3.1) 1986 (78.0) 482 (18.9)	 37 (3.0) 1020 (82.5) 179 (14.5)
Family history [ł]	2768 (32.0)	1351 (29.2)	939 (34.6)	478 (37.0)*
Overweight, n (%)	2097 (24.0)	1178 (25.1)	641 (23.4)	278 (21.3)#
Obese, n (%)	4865 (55.7)	2403 (51.2)	1632 (59.5)	830 (63.6)*
Abdominal Obesity, n (%) [ϕ]	4003 (58.4)	1870 (53.8)	1444 (61.6)	689 (66.7)*
Hypertension, n (%) (n=8679)[§]	4819 (55.5)	2353 (50.4)	1593 (58.5)	873 (67.7)*
	Mean ± SD			
Age (Years)	45.7 ± 5.0	45.4 ± 5.1	45.8 ± 4.6	46.8 ± 4.9**
Body mass index (kg/m^2)	25.5 ± 3.4	25.2 ± 3.3	25.8 ± 3.3	26.3 ± 3.5**
Waist circumference (cm)	91.1 ± 8.0	90.1 ± 7.8	91.7 ± 7.9	93.1 ± 8.3**
Blood Pressure (mmHg)				
Systolic	126.7 ± 16.2	125.2 ± 15.5	127.2 ± 16.4	130.8 ± 17.3**
Diastolic	84.1 ± 10.9	82.9 ± 10.7	85.1 ± 10.6	87.1 ±11.3**

Legend: Trend χ^2 square- * p<0.0001, # p=0.011, One Way ANOVA - ** -p<0.0001
a: occupation data available for 8148 individuals; ł : data available for NGT: 4633; IGT: 2715; DM: 1293; [§:] data available for NGT: 4688; IGT: 2722; DM: 1289; [ϕ:] data available for NGT: 3476; IGT: 2345; T2DM: 1033; Overweight defined as: BMI 23.0 – 24.5 kg/m^2 Obesity defined as: BMI ≥ 25.0 kg/m^2; Abdominal obesity defined as: waist circumference≥ 90 cm; Hypertension: Blood pressure ≥ 130/85 mmHg on two occasions Ram J, Nanditha A, Selvam S. J Assoc Physicians India (2014)[169]

3.5.3 Prevalence of risk clusters stratified according to glycaemic status

Figure 3-2 shows the prevalence of risk clusters (blood pressure, hypertension and parental history of diabetes) stratified based on glycaemic status. In this cohort, risk factor clustering was not present in 8% of the screening participants (NGT: 10.1%; IGT: 6.2%; T2DM: 4%; P<0.0001). A clustering of risk factors (2 or more) was present in many individuals; the most frequent cluster was overweight / obesity (BMI ≥23 kg/m^2) and hypertension (22.9%). The BMI and hypertension cluster (31.1%) was most prevalent in this cohort whereas, clustering of family history of diabetes and hypertension was the least (2.4%). Clustering of 3 risk factors occurred in 15.1%. As expected, the prevalence of risk clusters increased with degree of hyperglycaemia, the percentage increased linearly from NGT, through IGT to diabetes. (NGT: 11.7%; IGT: 17.1%; T2DM: 23.1%; P<0.0001). Importantly the presence of positive family history of diabetes had a tendency to cluster with modifiable risk factors such as obesity and hypertension rather than to present in isolation (isolated family history: 3.1%;family history + BMI: 11.6%; family history of diabetes +hypertension: 2.4 family history of diabetes +BMI+ hypertension: 15.1%).

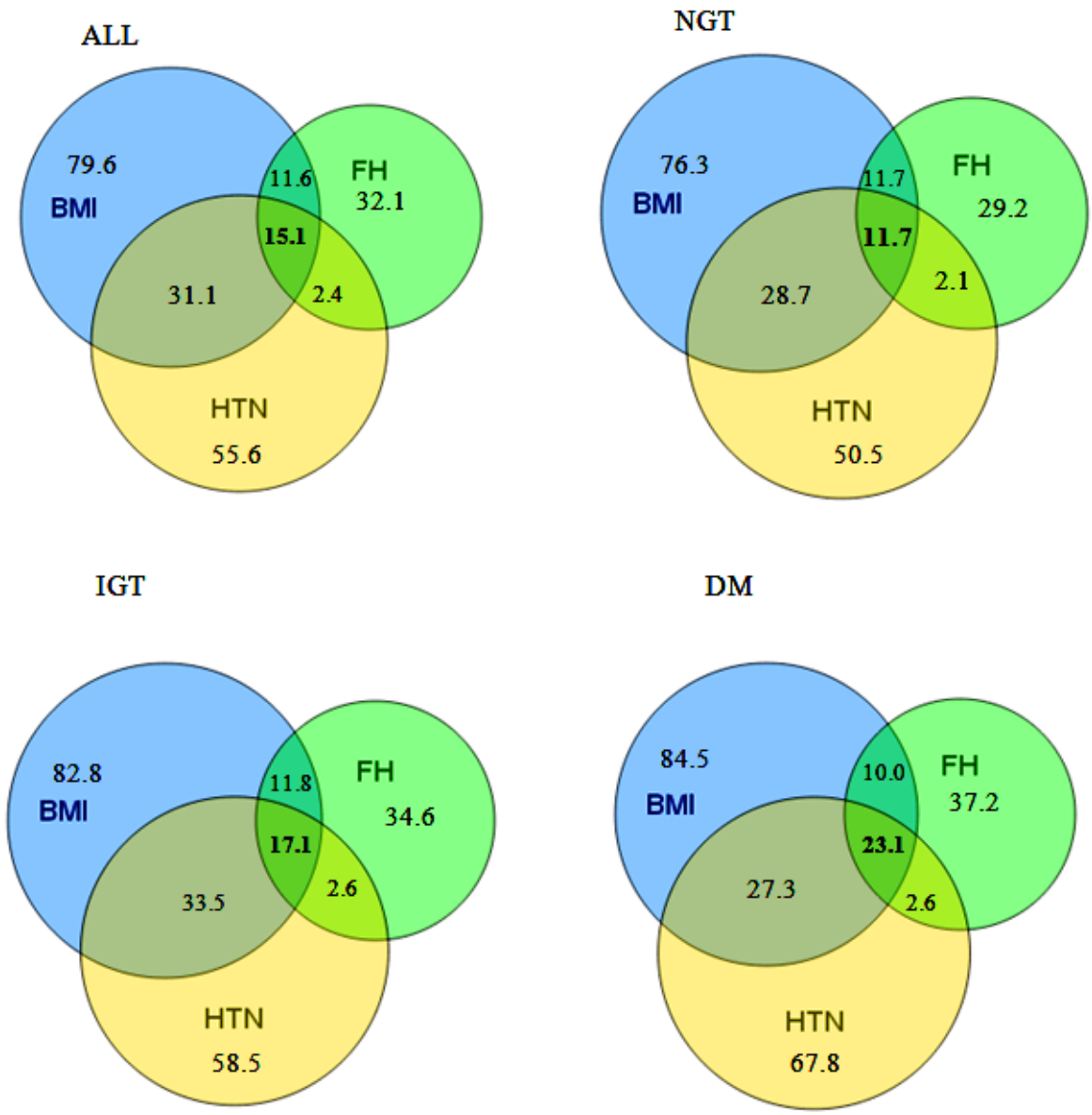


Figure 3-2: Clustering of diabetogenic risk factors (family history of diabetes, over weight and hypertension) according to glycaemic status

Legend: The figure shows the prevalence of risk factor clusters among the population screened, based on glycaemic outcomes. The presence of hypertension, overweight/ obesity and positive family history were observed in 11.0% of the study group. Newly detected diabetes cases had increased risk factor clusters as compared to NGT & IGT group (NGT:7.6%; IGT: 13.2; T2DM: 17.1%; P<0.0001).

Notes: FH: family history; BMI: body mass index; HTN: hypertension

3.5.4 Confirmatory test and recruitment

Of the 2744 (31.4%) eligible participants, we had, with exclusion of 912 individuals with 2-hr post glucose (2-hr PG) load <8.9 mmol/l (to enhance the yield for the second OGTT), invited 1832 (66.8%) individuals for the confirmatory test. Among them, 1369 (50%) agreed to a second OGTT. Of these, 580 (42%) had persistent IGT, of whom 43 (7%) did not wish to participate further, leaving 537 participants who were randomly assigned to the mobile phone messaging intervention (n=271) or to standard care (n=266), and were included in the primary analysis.

Baseline characteristics were similar between groups (**Table 3-2**). The study cohort sampling mostly comprised of skilled labourers (334 out of 537 (62.9%)) (**Table 3-2**). At entry, no participant in either group was receiving lipid modifying treatment, but 69 (26%) in the intervention group and 69 (25%) in the standard care group were taking medication for blood pressure reductions. Dietary energy intake, compatibility of diet with advice during the trial, and the distribution of physical activity scores were similar in both groups at baseline (**Table 3.2**).

Table 3-3: Baseline characteristics of study participants

Characteristic	Control (n=266)	Intervention (n=271)
Age (years)	46.1±4.6	45.9±4.8
Occupation		
Unskilled workers	11 (4.0)	9 (3.0)
Skilled	170 (64)	164 (61)
Executives	85 (32.0)	98 (36.0)
Family history of diabetes	131 (49.0)	150 (55.0)
Body mass index (kg/m^2)	25.8±3.0	25.8±3.3
Waist circumference (cm)	92.7±7.3	92.6±7.1
Blood Pressure (mmHg)		
Systolic	123.4±14.3	123.1±13.6
Diastolic	80.2±8.4	80.2±8.4
Receiving hypotensive drugs	69 (26)	69 (25)
Plasma glucose (mmol/l)		
Fasting	5.70±0.55	5.63±0.53
2-hr	8.90±0.86	8.79±0.78
Serum lipids (mmol/l)		
Total cholesterol	4.91±0.94	4.87±0.89
HDL cholesterol	0.90±0.19	0.90±0.21
Triglycerides*	1.6 (1.2-2.3)	1.6 (1.1-2.1)
HOMA-IR $\Delta I_{30-0}/G_{30}$ (pmol/mmol)	3.2±1.5 48.9 (27.9-78.5)	3.0±1.3 47.6 (30.0-81.7)
Dietary energy intake (kcal)	2100±278	2121±296
Baseline dietary healthy practice (%)	136 (51)	141 (52)
Physical activity score*	36 (31-56)	36 (27-54)

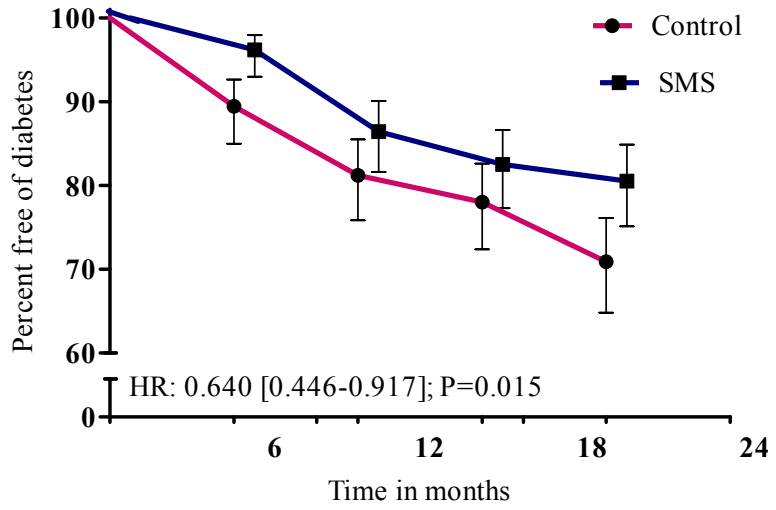
Legend: Data are mean ± SD; * Median (inter-quartile range). Homogeneity of the study groups was analyzed by two-tailed t-test.

Ramachandran A, Snehalatha C, Ram J, et al. Lancet Diabetes Endocrinol 2013[130].,

3.5.5 Study outcome analysis

3.5.5.1 *The primary outcome was incident type 2 diabetes*

At final follow-up, the response rate was 96% (n=517; **Figure-3.3**). The mean time (months) to incident diabetes was 20.9 [95%CI: 20.2-21.7] in the control group compared with 21.9[95%CI: 21.3-22.5] in the intervention group (P=0.011). Three patients (two [<1%] in the control group and one [<1%] in the intervention group) were diagnosed with T2DM by treating physicians outside the trial. These patients were included in the analysis after the diagnosis was confirmed from medical records. Other cases were ascertained during the trial. Including the three patients who had already been diagnosed, 50 (18%) men in the intervention group developed T2DM over the 2 years compared with 73 (27%) control patients (ARR: 9%; **Figure 3-3**).The intervention reduced the incidence of T2DM during the course of the study (β –0·447; HR: 0·640 [95% CI: 0·446–0·917]; P=0·015; **Figure 3-3)**. The number needed to treat to prevent one case of T2DM was 11 (95% CI 6–55).



Time (Months)	**0**	**6**	**12**	**18**	**24**
Subjects at risk (n)					
Control	266	228	215	176	187
Intervention	271	245	240	196	209

Figure 3-3: Proportion of individuals without diabetes during the study (Kaplan-Meir survival curve)

Legend: Error bars show 95% CIs. Diagnosis of diabetes was based on WHO criteria[2]. HR=hazard ratio;

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3.5.5.2 *SMS acceptability*

The frequency of mobile phone messaging at baseline was decided individually, in line with participants' wishes. Initially a median of 18 messages per month (range 8–24) were requested. At final follow-up, the median requested was 12 (range 8–16). Analysis of the acceptability questionnaire data showed that messages were generally welcomed, and the median questionnaire score out of 6 was 5 (range 3–6). No more than eight (3%) of 271 people at any review stated that receiving the messages was disturbing them.

3.5.5.3 *Secondary outcome analysis*

Secondary outcomes were BMI, waist circumference, systolic and diastolic blood pressure, lipid profile (total and HDL-C and triglycerides), total dietary energy intake, and physical activity score.

Linear mixed model analyses showed no significant effect of the intervention on BMI, waist circumference, blood pressure, or serum cholesterol and triglycerides, but there was a significant improvement in HDL-C in the intervention group compared with the control group (**Table 3-3**). Total dietary energy intake was lower in the intervention group than in the control group, whereas physical activity scores did not differ (**Table 3-3**). At the end of follow-up, a greater proportion of participants in the intervention group were adherent to diet than in the standard-care group (**Table 3-3**), but adherence to physical activity recommendations did not differ between the two groups.

One patient in the control group died suddenly at the end of the first year due to cardiac arrest.

Table 3-4: Secondary outcomes and adherence to dietary intake and physical activity recommendations at the end of follow-up

Variables	Baseline	6 months	12 months	18 months	24 months	Estimated marginal mean change	P value
Body mass index (Kg/m^2)							
Control	25.8±3.0	24.7±5.8	24.8±5.8	24.9±5.8	24.8±5.8	-0.05(-0.46 to 0.37)	0.828
Intervention	25.8±3.3	24.6±5.9	24.8±6.0	24.8±6.0	24.8±6.0		
Waist circumference (Cm)							
Control	92.7±7.3	92.5±7.8	92.4±7.8	92.7±7.8	92.8±7.7	0.04(-0.56 to 0.64)	0.897
Intervention	92.6±7.1	92.3±8.1	92.4±8.2	92.9±8.0	92.9±8.2		
Systolic blood pressure (mmHg)	123.4±14.3	122.4±13.4	120.8±12.8	120.6±12.1	119.5±12.0	0.04(-0.96 to 1.03)	0.937
Control	123.1±13.6	122.5±13.1	120.2±12.3	121.4±13.0	119.7±12.5		
Intervention							
Diastolic blood pressure (mmHg)	80.2±8.4	78.8±7.8	78.4±6.8	78.6±6.5	77.7±7.0	-0.07(-0.64 to 0.49)	0.796
Control	80.2±8.4	78.8±6.9	78.1±6.5	78.4±6.9	77.9±7.9		
Intervention							
Total cholesterol (mmol/l)							0.846
Control	4.91±0.94	---	4.90±0.9	---	4.8±1.0	0.01(-0.08 to 0.01)	
Intervention	4.87±0.89		4.90±0.9		4.9±0.9		
HDLC (mmol/l)							
Control	0.90±0.19	---	0.99±0.22	---	0.92±0.19	0.03(0.01 to 0.05)	0.003
Intervention	0.90±0.21		1.04±0.25		0.96±0.22		
Triglycerides (mmol/l)							
Control	1.6(1.2-2.3)	---	1.6(1.2-2.3)	---	1.6(1.2-2.3)	-0.08(-0.17 to -0.06)	0.050
Intervention	1.6(1.2-2.1)		1.5(1.2-2.1)		1.5(1.2-2.1)		
Dietary energy intake (kcal),							
Control	2100±278	2067±269	2033±266	2016±265	1993±259	-43.7(-65.5 to -22.0)	<0.0001
Intervention	2121±296	1984±286	1976±293	1971±288	1937±281		

Table 3-3: Secondary outcomes and adherence to dietary intake and physical activity recommendations at the end of follow-up (continued)

Variables	Baseline	6 months	12 months	18 months	24 months	Estimated marginal mean change	P value
Diet adherence, n(%)							
Control	136 (51.1)	140 (52.6)	152 (57.1)	155 (58.3)	143 (53.8)	1.357 [1.008-1.826]	0.0422
Intervention	141 (52.0)	179 (66.1)	166 (61.3)	171 (63.1)	184 (67.9)		
Physical activity							
Control	36(31-56)	39(23-52)	39(21-52)	39(25-56)	39(29-56)	–1·0(–2·0 to 0)	0.758
Intervention	36(27-54)	39(25-52)	39(25-52)	42(27-52)	42(28-56)		
Physical activity, n(%)							
Control	205 (77.1)	203 (76.3)	205 (77.1)	196 (73.7)	197 (74.1)	1.110[0.779-1.573]	0.572
Intervention	202 (74.5)	203 (74.9)	201 (74.2)	194 (71.6)	202 (74.5)		

Legend: Data are mean (SD) or median (IQR), unless otherwise indicated. We used mixed-linear regression analysis, taking into account visit and intervention, to generate estimated marginal means and difference in mean change (95% CI) at the end of follow-up.*Log-transformed data (by back transformation). †Logistic regression analysis with repeated measures taking into account visit and intervention to compare adherence to diet and physical activity between groups; BMI, WC, SBP, DBP, total energy intake and dietary adherence. Physical activity and adherence to the physical activity were measured at every 6 months; lipid profile (TC, TG and HD-C) were measured at annual visits.

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3.5.6 Predictors of diabetes

Among the baseline variables the significant predictors of incident T2DM were: a) raised 2 hr plasma glucose, b) increased insulin resistance , and c) lower insulinogenic index (**Table 3-4**). Improvement in dietary compliance was inversely associated with the development of T2DM and increase in BMI from baseline was detrimental. Significance of each variable was sustained on entry into a Cox proportional hazard model.

Table 3-5: Predictors of diabetes

Variables	HR [95%CI]	P value
Baseline 2-hr plasma glucose (mmol/l)	1.728 [1.406-2.122]	<0.0001
Baseline HOMA-IR	1.175 [1.075-1.285]	<0.0001
Baseline insulinogenic index (pmol/mmol)	0.993 [0.988-0.998]	0.006
Change in dietary practice improvement	0.482 [0.327-0.710]	<0.0001
Change in BMI (Kg/m^2)	1.329 [1.151-1.535]	<0.0001

Legend: change in BMI, diet adherence and physical activity were computed by subtracting final follow-up from the baseline values; for diabetic cases the values at the time of diagnosis considered as the final value.

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3.6 Predictors of normoglycaemia

Although reversal of prediabetes to normal glucose tolerance (NGT) is not uncommon, the effect of lifestyle intervention on reversal from persistent prediabetes to NGT have not been studied in detail in Asian Indian population. In view of its potential importance of achieving NGT with the lifestyle intervention, the consequences of early reversal to NGT were explored in detail. Also, there have only been a few studies on the cardiovascular risk factors during long term outcome of prediabetes.

The aims of this ancillary cohort analysis of a prospective, prevention study among Asian Indians, with IGT were: a) to quantify the risk reduction in incident diabetes during a 24 months follow-up in participants who achieved NGT at 6 months (NGT-6m) compared with those who remained in the prediabetic state; b) to study the factors influencing the reversal of prediabetes to NGT and c) to determine whether the cardiometabolicrisk factors improved among those who reverted to NGT at the end of the study.

Risk of T2DM in 2 year was lower by 75% in NGT-6m group (hazard ratio: 0·25[95%CI:0·12-0·52]). Predictive variables for reversal to NGT-24m were good baseline beta-cell function (OR:2.79[95%CI:2.30-3.40]) and its further improvement (OR:5.70[95%CI:4.58-7.08]), and NGT-6m(OR:2.10[95%CI:1.14-3.83]). BMI decreased in those who reverted NGT. Deterioration to T2DM resulted in increase in levels of cardiometabolic risk factors. In summary, early reversion to NGT by lifestyle intervention in prediabetic men was associated with significant reduction in subsequent incidence of diabetes. Good baseline beta-cell function and its further improvement, and NGT-6m were associatedwith reversion to NGT-24m. Reversion to NGT resulted in modest improvements while conversion to T2DM resulted in significant worsening of cardiometabolic risk profile.

The findings of this analyses has been accepted in diabetes care and the copy of the manuscript was enclosed as an appendix (appendix)

3.7 Mechanism of action of intervention

In prevention trials in western populations, the beneficial effects were largely attributed to weight reduction [111,112,170], whereas in Asian populations the benefit of lifestyle intervention was seen to be independent of weight loss [114,116]. The

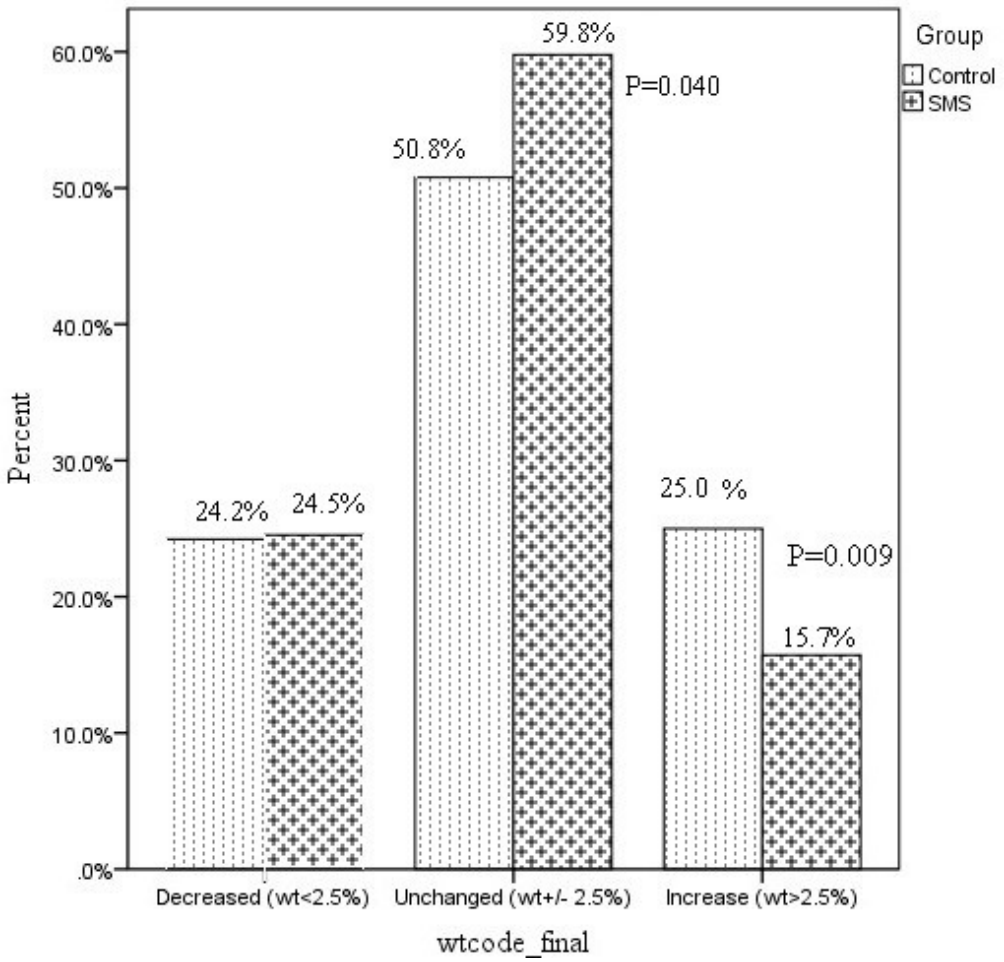
components of lifestyle intervention that are responsible for the reduced incidence of diabetes have not been studied in detail in Asian Indians. Therefore, the objective of this section is to unravel the mechanism of action of lifestyle intervention in Asian Indian men with prediabetes.

3.7.1 Effect of weight change on prevention of diabetes

As shown in **Table 3-3** there was no significant difference in BMI between standard care advice and intervention groups at the end of the study. It is worth mentioning that the baseline BMI in western diabetes prevention studies (Diabetes Prevention Programme (DPP)-mean BMI: 34.0 kg/m^2; Diabetes Prevention Study (DPS)-mean BMI: 31.3 kg/m^2) were much higher than that of the present study (mean BMI: 25.8kg/m^2; around 21.0% to 34% lower than that of the western studies). Hence, the western studies may demonstrate independent benefits of BMI on the incidence of diabetes. On the other hand it is hard to reconcile such benefit in this trial because of their lower BMI at baseline and primarily we were not advising participants to lose weight rather we were emphasizing healthy dietary habits and benefits of increased physical activity. The objective of this *post hoc* analysis is to find out the relationship between modest weight change and incident diabetes in this cohort of Asian Indian men. The statistical analyses performed in this exploratory analysis were adopted from the Prevention of Diabetes and Obesity in South Asians (PODOSA) study[171]. The participants were categorized into 3 groups based on their weight change at the end of the study:

- Group in which weight increased by 2.5% or more
- Group in which weight remained unchanged (changes < ±2.5%)
- Group in which weight decreased by 2.5% or more

Figure 3-4 : Changes in weight at the end of the study - stratified based on study groups



Legend: P value computed by χ^2 test; categories are based on: unchanged: <± 2.5% weight change from baseline; decrease of 2.5% or more weight change from baseline; increase of 2.5% or more weight change from baseline. Percent weight loss was calculated using the formula: ((weight (Kg) at final) – (weight (Kg) at baseline) / (weight (Kg) at baseline)*100).

3.7.2 Effect of healthy lifestyle goals on prevention of diabetes

The changes in dietary habits and regular physical activity at the end of the study are shown in the **Table 3-6**. A higher percentage of persons in the intervention group reported changes in dietary habits compared with the control group. There was no difference in levels of physical activity or in change in BMI between the study groups. Univariate logistic regression analysis showed that among the goals achieved the greatest protection against development of diabetes was achieved by a reduced BMI of at least 1 unit (OR: 0.15 [95%CI: 0.01-0.47]). Among the dietary factors, reduction in portion size (OR: 0.39 [95% CI: 0.25-0.60]), reduction in oil intake (OR: 0.46 [95% CI: 0.30-0.69]) and reduced consumption of carbohydrates (OR: 0.52 [95% CI: 0.34-0.78]) all had inverse associations with diabetes. Improvement in the physical activity did not show any association with incident diabetes. The proportion of individuals who achieved ≥ 3 lifestyle goals was higher in the intervention than in the control group (132 (50.6%) vs. 105 (41.0%); P=0.028).

Table 3-6: Number (%) of study individuals showing improved diet habits and regular physical activity at the end of the study

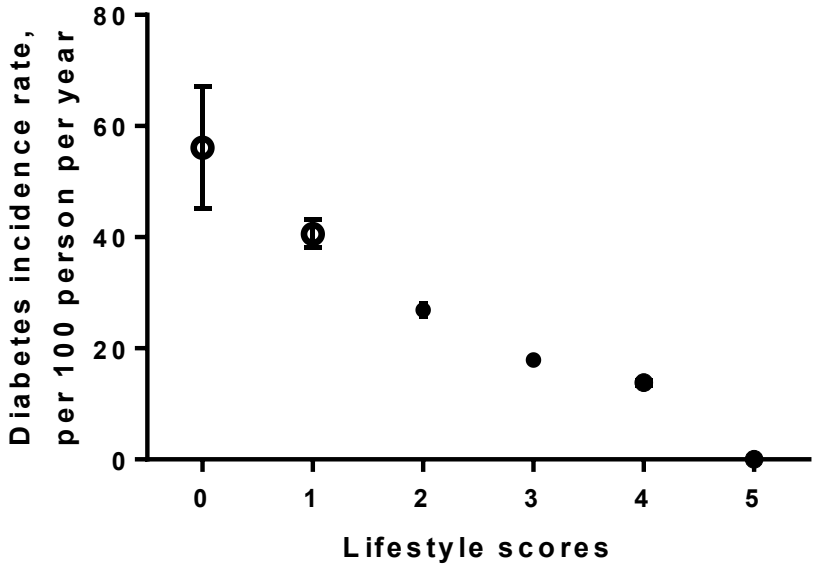
Variables n (%)	Control (n=256)	Intervention (n=261)	P value *	Diabetes incidence n(%)	OR (95 % CI) (all individuals)
Decreased consumption of carbohydrates **	121 (47.3)	149 (57.1)	0.025	49(18.1)	0.52 (0.34-0.78)
Decreased portion size**	107 (41.8)	137 (52.3)	0.015	37(15.2)	0.39 (0.25-0.60)
Decreased consumption of oil intake **	124 (48.4)	162 (62.1)	0.002	50(17.5)	0.46 (0.30-0.69)
Decreased BMI of at least 1 unit (Kg/m^2)	34 (13.3)	27 (10.3)	0.301	3 (4.9)	0.15 (0.01-0.47)
Regular physical activity §	197 (76.9)	202 (77.4)	0.899	97(24.3)	1.14 (0.70-1.89)

Legend: *: P values were determined by the χ^2 test between the two groups;
**:Nutrient intakes were calculated from diet questionnaire
§ : Exercise habits were assessed by self-reported physical activity questionnaire; OR: odds ratio; CI: confidence intervals.

3.7.3 Association of Lifestyle goals with diabetes

There was a strong inverse correlation between the success score and the incidence of diabetes as shown in the **Figure 3-4**. Among the 15 individuals who did not achieve any of the lifestyle goals (control: 12; intervention: 3), 8 (53%) developed diabetes. None of the individuals who achieved all the five lifestyle goals (control: 5; intervention group: 13) developed diabetes.

Multivariable logistic regression analysis adjusted for baseline values of age, BMI and 2-hr PG showed that individuals who achieved ≥ 3 goals were at reduced risk of developing diabetes (odds ratio: 0.30 [95% CI: 0.19-0.48]; P<0.0001) compared with the individuals who achieved <3 goals.



Variables	Zero	One	Two	Three	Four	Five
DM case	8	39	42	23	11	0
Total	15	101	164	135	84	18

Figure 3-5: Diabetes incidence rate by lifestyle goals (number of intervention goals achieved at year 2).

Legend: Intervention and control groups were combined for this analysis;
Error bars show 95% CIs. Diagnosis of diabetes was based on WHO criteria[2].
HR=hazard ratio.

3.8 Discussion

The present trial is the first to demonstrate benefit of SMS as a tool in the prevention of T2DM. The 36% reduction in progression to diabetes is similar to that reported previously in India using personal contact methods by our group[116]. The participants included in the trial were at especially high risk, having 2-hr PG levels ≥8.9mmol/l in their first OGTT. Their high rate of progression to diabetes, at 30%, was similar to that reported in our earlier Indian diabetes prevention programme [116]. The text messages were generally well accepted by most of the participants. Therefore, this study provides initial evidence that text messaging can be an effective and acceptable tool for lifestyle modification to reduce diabetes incidence. Though, short-term studies showed effectiveness of SMS in diabetes management [172-176], asthma [177,178], smoking cessation [149] and adherence to the medication and reminders for outpatient appointments [153,179,180], to our knowledge this is the first long-term trial to demonstrate the effectiveness of SMS in diabetes prevention. In fact, the effect of intervention was significant during the first 6 months and was sustained for over 2 years.

In this study, baseline 2-hr PG, baseline insulin resistance, increasing BMI during the follow-up were perilous whereas a high baseline early insulin secretion (insulinogenic index) and improved dietary compliance during the study were protective. Based on this finding, this analysis supports the hypothesis that progressive weight gain is an etiological factor before the onset of T2DM[181].

In this study, the effect of intervention was through the maintenance of weight and/or prevention of weight gain from occurring rather than from weight loss. The relationship between obesity and diabetes prevalence rates were a complex phenomenon and was appear to be modified by ethnicity. India, for instance, has a

very low prevalence of obesity than the U.S., but higher rates of T2DM [182]; besides that, in Asian populations increased risk of diabetes starts at a relatively lower BMI than that of Europeans[183]. The recently published UK bio bank study demonstrated that compared with whites with a BMI of 30 kg/m^2, South Asians had an equivalent prevalence of diabetes at a BMI of 22.0 kg/m^2 in women and 21.6 kg/m^2 in men, which is lower end of the normal BMI range for white populations[184]. Thus, even a modest amount of weight gain may be particularly detrimental to diabetes risk for Asians compared with other ethnic populations[185].

Furthermore, the present trial *post hoc* analysis to study the mechanism of benefit of intervention showed that the favourable outcome of reduced incidence of diabetes in the intervention group was associated with the increased compliance to the healthy lifestyle goals relative to the control group. The outcome of lifestyle modification was more pronounced among individuals who achieved ≥ 3 goals; on the other hand, the failure to make any changes resulted in an increased incidence of diabetes in either group. Amongst the dietary goals, the strongest association was observed with decrease in portion size (total energy intake) followed by decreased consumption of oil. Similar observations were also reported in the DPS [111], in the DPP [112] and in the Nurses' Health Study [186]. In these studies, reduction in incidence of diabetes was mainly attributed to the benefits of weight reduction which occurred due to the improvements in diet habits and in physical activity levels. In our study, a reduction in BMI had an independent protective effect, though the quantum of reduction was small and occurred only in small percentages in both groups. Therefore, the benefit of lifestyle intervention appeared to be largely related to improvements in diet habits which led to biochemical and behavioural improvement unrelated to weight loss.

In this study, the beneficial outcome of decreased incidence of diabetes was not associated with improved physical activity levels. In this study, we assessed physical activity by questionnaire only, a method that could have missed small changes. It is conceivable that activity information derived from self-report is potentially subject to response bias (e.g. imprecise recall, influence of social desirability) which is unavoidable in epidemiological settings. The beneficial effects of improved physical activity in the prevention of diabetes[111,112,114] was already established including previous trial in India[116] by our group. It is also to be noted that 64% of the cohort already had good levels of occupational physical activity at baseline. Therefore, objective measurements such as accelerometer are required to ascertain the beneficial changes of physical activity with incident diabetes.

HDL-C levels were slightly but significantly higher at the end of the trial in the intervention compared with the control group. This finding was analogous to the Oslo Diet and Exercise study[187] in high-risk individuals, which showed an improvement in HDL-C after diet intervention compared to control. Weight loss and increased physical activity are two positive determinants of circulating HDL-C but in our study we cannot be sure of the mechanism. Such small differences in HDL-C have been reported previously in conventional diabetes prevention studies and may well have cardio-protective effects[111,188], and anti-inflmmatory and anti oxidant properties[189].

3.9 Limitations

Our study has some limitations. Firstly, the trial included only working men. Hence, the efficacy and acceptability of SMS among women, and amongst other groups of men, need to be addressed. Secondly, the setting was an urban population in India. The applicability of text messaging to other populations, such as those living in rural areas, needs to be studied. Thirdly, we did not objectively measure the beneficial

improvements of physical activity levels in this study cohort. In our latest prevention trial which is presently commenced we rectified most of those limitations (study sample mostly comprised of semi-urban population; number of women participants: 30%; actigraph as an objective measure of physical activity improvements). Nevertheless, the proof of principle has been established that automated SMS delivery is a practical and successful tool for education and motivation of people with prediabetes.

3.10 Comments

By 2025, it is estimated that 70% of the Asian Indians will be of working age group and this would make India—by an order of magnitude—the largest single positive contributor to the global workforce over the next three decades [190]. The prevalence of undetected diabetes among industrial workers in Asian Indian individuals is very high[191]. Furthermore, IDF predicted that there are around 65.1 million individuals with prediabetes in India with the potential to develop diabetes at a later stage[1]. Hence, it is important to identify pragmatic, innovative and scalable strategies to prevent or postpone lifestyle diseases such as diabetes and obesity. Our results provided initial and important evidence that motivation through SMS may be a highly effective and acceptable tool to substantially reduce incidence of diabetes. According to our findings, the lifestyle intervention through mobile phone based text messaging was effective, with one case of diabetes prevented by treating 11 persons with prediabetes for two years. Text messaging may now become part of an alternative strategy to disseminate healthy lifestyle principles effectively in the field of preventive medicine.

4 PREDICTORS OF TYPE 2 DIABETES – CLASSICAL RISK FACTORS

4.1 Predictors of diabetes

The putative predictors of type 2 diabetes (T2DM) such as glycaemic measures and surrogate insulin measures were already explained in detail in **Chapter-1**. In this chapter the findings of the association of classical risk factors with diabetes are presented.

4.2 Research objectives

The main objectives of this chapter are to investigate simple, inexpensive, accurate markers which could predict incidence of T2DM. The markers explored in this chapter relate primarily to elucidation of the pathogenesis and mechanisms of disease and demonstrate that a prediction model based on the pathophysiology of the disease performs superiorly to other clinical models in predicting the risk of future T2DM. The overview of approaches to clinical predictors for T2DM is depicted in **Figure 4-1**. Dysregulation of many biological pathways leads to the development and progression of T2DM[120]. According to the current understanding of the pathogenesis of diabetes there exist vicious circles which link insulin resistance and beta-cell dysfunction through hyperglycemia, hyperlip-idemia, glucotoxicity and lipotoxicity [192].

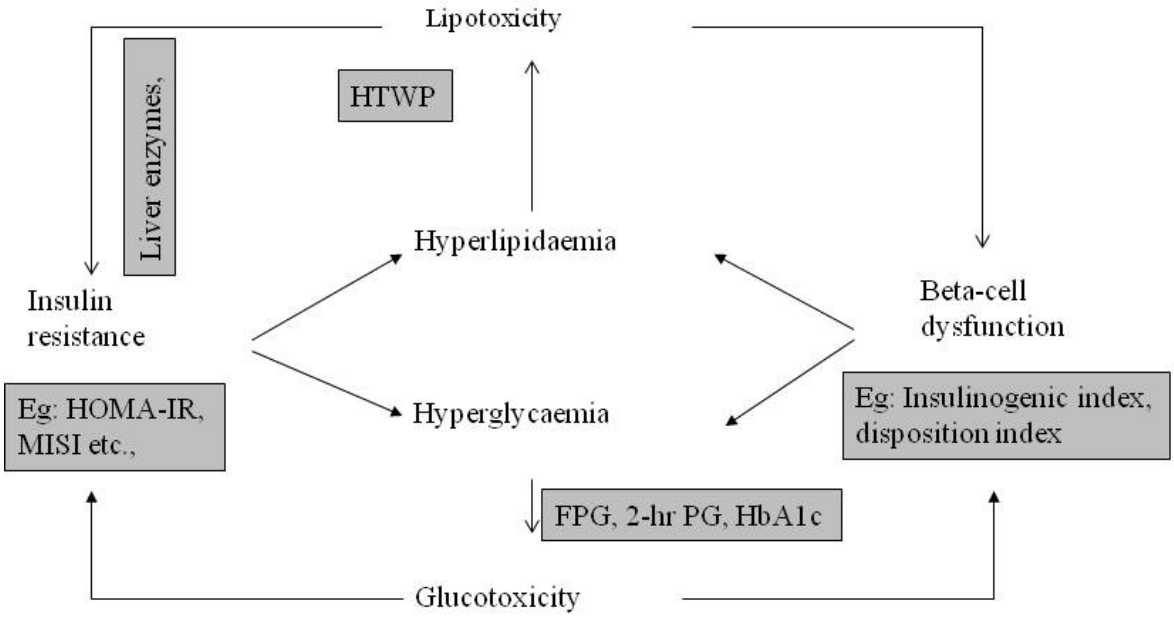


Figure 4-1: Approaches to clinical predictors for type 2 diabetes

Legend: GGT: Gamma-glutamyl transferase; ALT: alanine transaminase; HTWP: hypertriglyceridemic waist phenotype; FPG: fasting plasma glucose; 2-hrPG: 2hr plasma glucose after glucose load (75gms); HbA1c: glycosylated haemoglobin; HOMA-IR: Surrogate measure for insulin resistance. Figure adopted from Bonora (2008) [192]

4.3 Liver enzymes and diabetes

4.3.1 Rationale

'Insulin resistance syndrome' refers to the constellation of anthropometric and metabolic abnormalities such as abdominal obesity, glucose intolerance, hypertension and dyslipidemia[193]. Nonalcoholic fatty liver disease (NAFLD) refers to the hepatic manifestation of insulin resistance syndrome[194-197]. The liver, a major site of insulin clearance[198], plays an important role in maintaining fasting and postprandial glucose homeostasis[199]. Unlike in the skeletal muscle, where there may be extra cellular storage, all triglycerides are stored intra-cellularly in the liver. Hence, hepatic fat accumulation can adversely affect major aspects of hepatic metabolism, including insulin clearance and hepatic glucose output, with associated derangements of

intermediary metabolism. It has been suggested that hepatic dysfunction resulting from the insulin resistance syndrome and NAFLD may contribute to the development of T2DM[200]. Evidence from several large prospective epidemiological studies in multi-ethnic populations provides a strong link between elevated liver enzymes such as gamma-glutamyl transferase (GGT) [201-212] and alanine transaminase (ALT) [205,206,211,213,214] with development of diabetes.

There are, however, several shortcomings in the current evidence base: firstly, incident diabetes has been generally determined from self-reported information in medical records or the diagnosis was based on single fasting glucose measurements [202,207,211,212]. This could lead to potential misclassification bias. Furthermore, except two studies[205,211], other studies did not adjust for confounding variables such as insulin resistance, 2-hr plasma glucose (2-hr PG) and HbA1c. Hence, we have studied the association of GGT with incident diabetes, ascertained in a prospective epidemiological setting, after adjusting for a comprehensive array of covariates known to be associated with diabetes risk.

4.3.2 Methods

As described in detail in **chapter-2**, the underlying study design comprised 517 individuals with impaired glucose tolerance (IGT) at baseline. Since, both the control and intervention groups received one- to -one lifestyle advice at baseline, and with the major objective to explore the association of GGT with incident diabetes, we considered the cohort as a single group for this analysis.

4.3.3 Statistical analysis

Descriptive statistics of baseline measurements stratified based on glycaemic status, were analyzed by one way ANOVA with Bonferroni posthoc correction. Within group differences in GGT levels stratified based on glycaemic categories were assessed

using paired t-test. Cox's proportional hazard models[161,162] were computed to assess the relative risk of incident diabetes according to GGT activity: model-1, unadjusted GGT activity (dichotomized based on the median activity), model-2, adjusting for age, body mass index (BMI), family history of diabetes, smoking and drinking; model-3, included model-2 plus ALT activity; and model-4, included model-3 plus 2-hr PG, HbA1c, triglycerides, HOMA-IR (measure of insulin resistance) and insulinogenic index (measure of β-cell function). In order to determine the predictive power of baseline GGT in predicting diabetes, non-parametric, receiver operating characteristic (ROC) analyses were performed with the cumulative incidence of diabetes at the end of 2 years as the outcome variable [215]. The ROC is one of the most commonly used statistics, and is defined as a plot of sensitivity on the vertical axis against the plot of specificity on the horizontal axis. The value of ROC ranges from 0 to 1. A value of 0.5 equates to chance, and a value of 1 indicates perfect discrimination [215]. The area under the curve (AUC) of the ROC and a 95% confidence interval (CI) were calculated by the Delong method to evaluate the diagnostic utilities of the marker for diabetes prediction [216]. The optimal cut-off point of each marker was estimated by calculating the Youden index [217]. Specificity and sensitivity were calculated using that cut-off. The positive likelihood ratio was calculated using the formula: sensitivity / (1-specificity). A test for the equality between two AUC's was evaluated using an algorithm suggested by DeLong et al [216].

4.3.4 Results

4.3.4.1 *Baseline characteristics of the study participants according to glycaemic status at the end of the study*

Table 4-1 shows the baseline anthropometric and metabolic risk factors, according to OGTT-status at the end of the study. Individuals who developed T2DM had higher baseline fasting and 2-hr PG compared with regressors (IGT to normal glucose tolerance (NGT)). Also, the mean fasting plasma insulin was higher and the mean of OGTT-induced 30 minutes plasma insulin was lower in the progressors group compared with the regressors group.

Table 4-1: Baseline characteristics of study individuals based on the glycaemic outcomes at the end of 2nd year.

Variables	IGT to NGT (n=170)	IGT to IGT (n=224)	IGT to T2DM (n=123)
Age (Yrs)	46.1±4.8	46.1±4.7	46.1±4.5
Body mass index (Kg/m^2)	25.9±3.2	25.5±2.9	26.1±3.6
Waist circumference (Cm)	93.0±7.4	91.9±6.8	93.0±8.0
Blood pressure (mmHg) Systolic Diastolic	 123.8±12.8 80.1±8.0	 122.0±14.0 79.6±8.4	 124.0±14.4 81.2±9.0
Family history of diabetes, n (%) [a]	82 (48.2)	122 (54.5)	69 (56.1)
Smoking, n (%)[a]	37 (21.8)	44 (19.6)	36 (29.3)
Drinking habits, n (%)[a]	60 (35.3)	84 (37.5)	47 (38.2)
Glucose (mmol/l) Fasting 2-hr	 5.5 ± 0.5 8.5 ± 0.7	 5.6 ± 0.5 8.7 ± 0.8*	 5.8 ± 0.5†,¶ 9.2 ± 0.9†,¶
HbA1c (%)	6.0±0.3	6.1±0.3	6.3±0.4†,¶
Insulin (pmol/l)[b] Fasting Min 30 2-hr	 81.3 (57.8-102.6) 620.2 (425.4-957.7) 848.0 (581.9-1389.2)	 80.0 (58.3-99.7) 531.8 (350.3-809.3) 857.4 (583.6-1255.4)	 93.2 (72.2-118.1) *, ‡ 468.0 (338.9-832.7) *, ‡ 847.3 (604.4-1286.0)
Gamma-glutamyl transferase [b]	23.0 (17.9-33.8)	23.0 (17.4-35.5)	30.1 (22.6-48.8) †
Alanine transaminase	14.7±9.9	14.6±7.2	16.5±9.4

Legend: Data are mean±SD (for normally distributed variables) and analyzed by oneway ANOVA;
a: expressed as counts (percentage) for categorical measures – analyzed by χ test;
b: expressed as median (inter quartile range) for skewed variables – analyzed by Kruskal Wallis test.
* P< 0.05 vs. NGT; † P<0.001 vs. NGT; ‡ P< 0.05 vs. IGT; ¶ P<0.001 vs. IGT;

4.3.4.2 *Serum GGT activity according to glycaemic status at the end of the study*

During the 2 year follow up, there were 123 incident cases of diabetes among 505 non-diabetic men in this cohort. Mean age and BMI were 46.0 ± 4.7 years and 25.8±3.1 kg/m^2 respectively. The median values of serum GGT and ALT were 24.0 (inter quartile range (IQR: 18.0-36.1)) UL^{-1} and 13.0 (IQR: 10-19) UL^{-1} respectively. 38.0% of the study individuals consumed alcohol regularly and 23.6% were current smokers. The levels of GGT were significantly higher in those who developed diabetes at the end of the study (IGT to NGT: 23.0 (17.9-33.8) UL^{-1}; IGT to IGT: 23.0 (17.4-35.5) UL^{-1}; IGT to T2DM: 30.1(22.6-48.8) UL^{-1}; P<0.0001).The levels of ALT did not differ statistically between the groups (NGT: 14.7±9.9 UL^{-1}; IGT: 14.6±7.2 UL^{-1}; T2DM: 16.5±9.4 UL^{-1}, P=0.101). Hence, for the subsequent analysis, we studied only the effect of GGT on the incidence of diabetes.

4.3.4.3 *GGT activity at the end of the study*

In the total group, over the two years, the median GGT activity did not change significantly (final: 25.0 (19.0-37.0) UL^{-1}). However, changes were noted when they were categorized based on the final glycaemic outcomes. The mean change in GGT activity was significantly negative (95% CI <0) in individuals who reverted to NGT, whereas it was significantly positive (95% CI >0) in individuals who developed diabetes. There was no change in GGT levels in individuals who remained as IGT (mean change (95%CI) NGT: -3.5 (-6.4 to -0.6); IGT: -0.3 (-3.0 to 2.4); T2DM: 8.3 (3.6 to 13.0) UL^{-1}; P<0.0001).

4.3.4.4 *Correlates of GGT*

We observed significant, positive, univariate relationships between baseline GGT and triglycerides, total cholesterol, ALT, HbA1c and HOMA-IR (P< 0.0001). Present alcohol consumption and obesity measures (BMI and waist circumference) were also correlated with baseline GGT levels (P<0.01). Hemodynamic measures and High Density Lipoprotein (HDL-C) did not correlate significantly with GGT levels.

4.3.4.5 *GGT and diabetes*

Figure 4-2 shows the association of GGT with incident diabetes. In model-1, GGT activity greater than or equal to the median of 24 UL^{-1} was associated with a significantly increased risk of diabetes (HR: 2.23 [95%CI: 1.53-3.25]; P<0.0001). In model-2 and model-3, the association of GGT remained unchanged even after the adjustment for study group, age, BMI, family history of diabetes, smoking, drinking and ALT (HR:2.14 [95%CI: 1.45-3.15]; P<0.0001). However, when GGT was further adjusted for 2hr PG, HbA1c, triglycerides, HOMA-IR and insulinogenic index, the relationship became somewhat weaker (HR: 1.75 [95%CI: 1.75-2.65]; P=0.006).

ROC analyses showed that the optimum discrimination of diabetes risk over the two-year follow-up period by baseline GGT activity was achieved with a cut-off of ≥ 26.4 UL^{-1} (AUC ROC [95% CI]: 0.637 [0.581-0.692]; P< 0.0001; sensitivity: 61.2%; specificity: 60.9%).

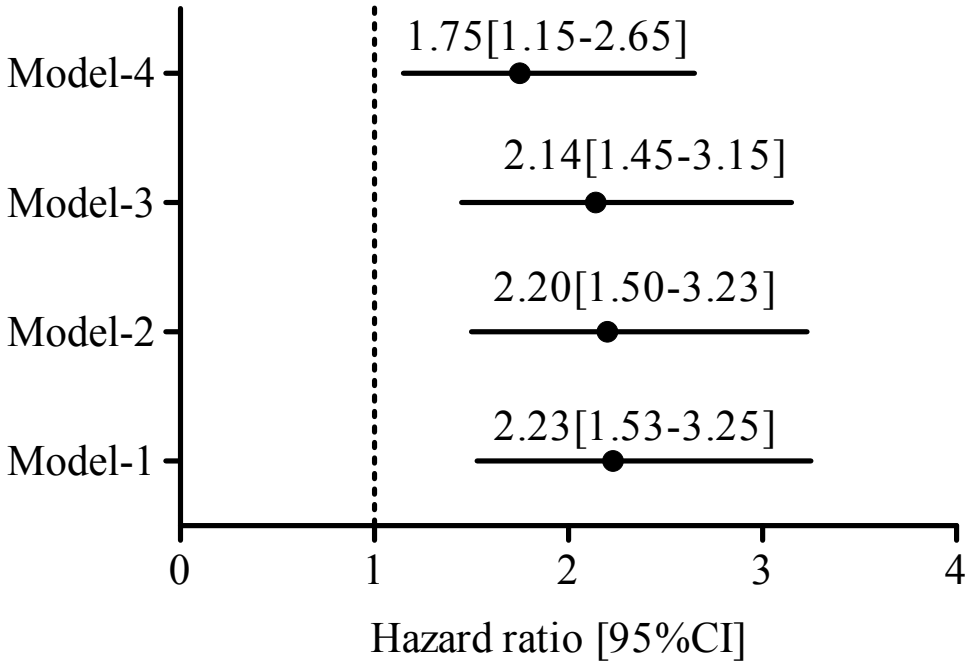


Figure 4-2: Cox proportional hazard showing predictive power of baseline GGT (in medians)

Legend:

Dependent variable: incident diabetes vs. non-diabetes; GGT values are dichotomized into below median (<24 UL^{-1}) and above median (≥ 24 UL^{-1}) values.

Model-1: Unadjusted model.

Model-2: adjusted for study group, age, BMI, family history of diabetes, smoking and drinking

Model-3: model-2 adjusted for ALT

Model-4: model-3 adjusted for 2-hr PG, HbA1c, TG, HOMA-IR (measure of insulin resistance) and insulinogenic index (measure of β- cell function).

4.3.4.6 *Discussion*

Elevated GGT activity in prediabetic individuals was associated with increased risk of developing diabetes even after adjustment for potential confounding variables. GGT activity above the median of 24.0 UL^{-1} were predictive of incident diabetes. The optimum threshold of ≥26.4 UL^{-1} in the ROC analysis was much lower than that of the conventional upper limit of normal (50 UL^{-1}) for GGT[218]. Revision of the current normal concentration range may be warranted if GGT is to be used in prediction of diabetes. Previous large, epidemiological, prospective studies demonstrated an association between elevated GGT levels and risk of diabetes in normoglycaemic individuals among white [211,219,220], Black [221] Japanese [222] and Korean populations [194]. The present analysis provides new evidence about the link between GGT and incident diabetes in Asian Indian individuals with IGT. A systematic meta-analysis showed that a 1U/l increase of natural logged GGT was associated with a 92% increase in diabetes risk (HR: 1.92 [95%CI: 1.66 –2.21]) in variably adjusted model [219]. This consistent evidence across all ethnic populations suggests this simple marker may be a useful test to identify individuals at high risk of diabetes.

No independent association between raised ALT levels and incident diabetes was seen in our population. This finding was in accordance with the findings from some [206,223,224] but not all [205,206,214,225] previous studies.

In this cohort of individuals with IGT, plasma GGT levels decreased in those individuals who regressed to NGT, whereas they increased in those who progressed to diabetes. This finding is in accordance with the previous prospective studies which showed a positive association of increases in GGT with T2DM [220], metabolic syndrome [226] and cardiovascular disease [227].

Increased GGT is conventionally interpreted as a marker of excessive alcohol consumption[228]. This relationship did not explain the association between GGT and diabetes seen in this cohort. The association of GGT with diabetes could be due to the excessive lipid accumulation in hepatocytes[229,230] and the resultant hepatic insulin resistance, possibly related to decreased portal insulin extraction[231] and increased glucose output, thereby contributing to the development of total body insulin resistance[230] and diabetes[232]. However, in this study the association of GGT with incident diabetes was independent of insulin resistance as measured by HOMA-IR. Therefore, the association of GGT with diabetes could be through pathways other than hepatic insulin resistance. Furthermore, GGT plays a pivotal role in extracellular catabolism of antioxidant glutathione[233,234] and is an early marker of sub-clinical inflammation [235]. In addition to its role in diabetes, elevated levels of GGT within its reference range are associated with metabolic syndrome [236,237] and cardiovascular mortality [238-240] and it considered as a proatherogenic marker because of its indirect relationship with biochemical steps that lead to low-density lipoprotein cholesterol oxidation [241,242].

Another, possible explanation is that, even mild chronic dysglycaemia (as in IGT in this cohort) is associated with oxidative stress[243], and raised levels of GGT might synergistically damage intracellular systems through excessive oxidative stress [235,242,244] which might play a role in causation and development of diabetes. In summary, we have demonstrated a positive association between GGT with incident diabetes in this cohort.

4.4 Association of hypertriglyceridemic waist phenotype with incident diabetes

4.4.1 Study rationale

Visceral adiposity precedes the development of diabetes[245,246]. There is strong evidence that excess of visceral fat, measured by enlarged waist circumference, is closely associated with insulin resistance and disturbances in lipid and glucose metabolism[247]. Previous studies in South Asians [134], including our study [248], have reported an association of waist circumference with diabetes, suggesting that the increased accumulation of fat in the abdominal cavity may be one of the contributors to diabetes in this ethnic group. The increasing prevalence of central-obesity, sedentary behaviour and unhealthy dietary habits leads to hypertriglyceridemia which is a risk factor for insulin resistance, T2DM and prediabetes [249]. Enlarged waist circumference cannot be distinguished from intra-abdominal adiposity and subcutaneous fat depots [250]. Therefore, Lemieux et al [251] proposed the presence of elevated triglyceride levels in the presence of central adiposity as a "proxy" measure to identify individuals with an increased risk of the atherogenic lipid triad (increased triglyceride levels, decreased HDL-C concentrations and the presence of small, dense LDL particles), hyperinsulinemia and sub clinical inflammation. In fact, the hypertriglyceridaemic waist phenotype (HTWP) was proposed as a simple screening method to identify participants at high cardiometabolic risk[252,253]. Though cross-sectional studies[254-257], including ours[258], had demonstrated an association of the phenotype and diabetes, the utility of this measure for the prediction of incident diabetes has not been studied much. This ancillary cohort analysis was done with the objective of analyzing the possible association of HTWP with incident diabetes considering the simplicity of identifying the presence of HTWP and its association with other cardiometabolic risk factors.

4.4.2 Methods:

The characteristics of the study participants and methods were already explained in detail in **Chapter-2**. Briefly, of the 537 individuals recruited for the study, 517 individuals responded till the final follow-up (response rate: 96.3%) out of which 123 individuals developed diabetes. The present analysis was limited to those individuals who completed the final year follow-up.

4.4.3 Definition of variables

The cut-off values for the waist circumference and triglycerides recommended for the Asian population by the International Diabetes Federation [6] were used to categorize the participants to four groups:

- Normal waist-line and triglycerides level (NWTG): waist circumference <90 cm; serum triglycerides <1.7 mmol/l
- Enlarged waist-line and normal triglycerides level – Isolated enlarged waist (iEW): waist circumference ≥90 cm; serum triglycerides < 1.7 mmol/l
- Normal waist-line and raised triglycerides level – Isolated hypertriglyceridemia (iHTG): waist circumference <90 cm; serum triglycerides ≥ 1.7 mmol/l
- Hyper triglyceridemic waist phenotype (HTWP): waist circumference ≥90 cm; and serum triglycerides ≥ 1.7 mmol/l.

4.4.4 Statistical analysis

General characteristics of individuals among the study phenotypes were compared by one way ANOVA for normally distributed variables following Bonferroni post hoc correction, Kruskal Wallis test for skewed variables with Dunn's multiple comparison test and $\chi 2$ test for categorical measures after with Bonferroni P value correction. Linear regression analyses were used to assess associations of iEW, iHTG and HTWP

with insulin resistance as measured by HOMA-IR (dependent variable). Adjusting for age, BMI, family history of diabetes, hypertension, fat%, smoking and alcohol consumption was performed in model-1 and in model-2 additionally for 2-hr PG and HDL-C. Cox regression analysis was used to calculate HR and corresponding 95% CI for the risk of T2DM for different categories of enlarged waist and/or hypertriglyceridemia as shown above after adjusting for potential confounding factors known to affect the outcome variable. Cox's proportional hazard models were computed to assess the relative risk of HTWP with incident diabetes after adjusting for the potential confounding variables. The covariates which showed significant univariate associations with HTWP were chosen for the multivariate analyses. Age, family history of diabetes and 2-hr PG values were included in the model due to their strong associations with diabetes. Analyses were adjusted for the dichotomous variables: study group, family history, known hypertension, smoking and drinking habits and for the continuous variables: baseline age, BMI, fat%, 2hr PG, GGT and HOMA-IR. Survival curve was computed based on the phenotype categories. $P < 0.05$ was considered statistically significant. SPSS (IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp) was used for the analyses.

4.4.5 Results

4.4.5.1 *The prevalence of isolated enlarged waist, isolated hypertriglyceridaemia and hypertriglyceridaemic waist phenotype*

Baseline characteristics of the participants classified on the basis of waist circumference and triglycerides levels are shown in Figure 4.2. iHTG was present in 64 (12.4%), 167 (32.3%) had HTWP and a total of 231 (44.7%) had fasting hypertriglyceridemia. Central adiposity was present in 358 (69.2%) participants amongst whom 191 (36.9%) had the iEW phenotype.

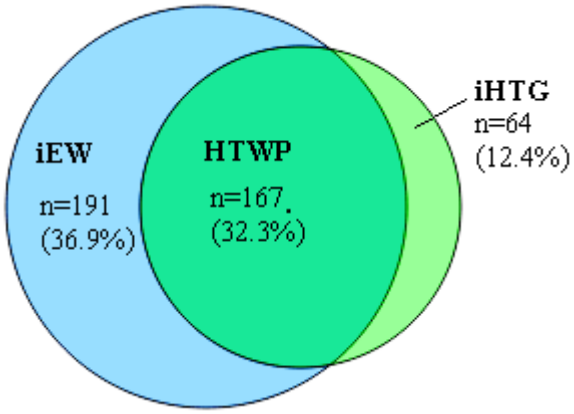


Figure 4-3: The prevalence of single abnormality (iEW and iHTG) and combination of both (HTWP)

Legend: iEW: isolated enlarged waist; HTWP: hypertriglyceridemic waist phenotype; iHTG: isolated hypertriglyceridemia.

4.4.5.2 *Baseline characteristics of four sub-groups*

The baseline characteristics of the four sub-groups are presented in **Table 4-2.** Participants with iEW and HTWP categories had significantly higher BMI, waist circumference and fat% compared with NWTG and iHTG groups (P<0.0001). Participants with HTWP had raised diastolic blood pressure compared with NWTG (P<0.05). No significant differences in fasting and 2-hr PG levels were observed between the groups. HTWP and iHTG phenotypes had significantly higher total cholesterol, and lower HDL-C levels compared with NWTG and iEW groups (P<0.0001). The HTWP group had higher insulin resistance compared with NWTG, iHTG (p<0.0001 for both) and iEW (P<0.05) phenotypes. The participants with HTWP also showed an increased insulinogenic index compared with NWTG (P<0.05).

Table 4-2: Anthropometric and clinical characteristics of individuals based on the categories of waist and triglycerides levels.

Variables	Total	NWTG	iEW	iHTG	HTWP
n (%)	517	95 (18.4)	191 (36.9)	64 (12.4)	167 (32.3)
Age (years)	46.1±4.7	46.2 ± 4.8	46.0 ± 4.8	45.3 ± 4.4	46.4 ± 4.5
Body mass index (Kg/m^2)	25.8±3.2	23.5 ± 2.3	26.8 ± 2.7 †, ¶	23.8 ± 2.7	26.8 ± 3.2†, ¶,**
Waist circumference (cm)	92.6±7.3	85.2 ± 3.6	95.8 ± 5.4 †, ¶	84.9 ± 3.2	96.4 ± 6.4†, ¶,**
Total body fat (%)	27.5 ± 4.7	24.6±4.5	28.6±4.4 †, ¶	26.1±4.3	28.3±4.3†, ¶,**
Blood pressure (mmHg)					
Systolic	123.1±13.7	120.6±13.3	124.3 ± 13.0	120.2 ± 12.9	124.2 ± 14.9
Diastolic	80.2±8.4	77.8 ± 8.4	80.6 ± 7.9 *	80.0 ± 6.8	81.1 ± 9.3*
Hypertension, n(%)	133 (25.7)	21 (22.1)	59 (30.9)	10 (15.6)	43 (25.7)
Family history n(%)	273 (53.3)	43 (45.2)	107 (56.9)	35 (54.8)	88 (53.4)
Smoking, n(%)	117 (22.7)	17 (17.6)	40 (21.3)	19 (26.8)	41 (26.2)
Drinking, n(%)	191 (38.0)	30 (30.2)	64 (35.5)	24 (40.2)	73 (41.8)
Plasma glucose (mmol/l)					
Fasting	5.62±0.54	5.57±0.54	5.63±0.53	5.54±0.52	5.67±0.56
2-hr	8.78±0.82	8.72±0.82	8.82±0.82	8.82±0.81	8.73±0.82
Lipid profile (mmol/l)					
Triglycerides	1.60(1.19-2.11)	1.20 (0.99-1.39)	1.23 (1.04-1.47)	2.35 (1.93-2.82)†,¶	2.19 (1.91-2.70)†,¶
Total cholesterol	4.87±0.91	4.67±0.66	4.63±0.88	5.09±1.02*	5.16±0.92†
HDL cholesterol	0.89±0.20	0.95±0.18	0.93±0.23	0.84±0.17 *	0.85±0.17*
Gamma-glutamyl transferase	32.6±25.5	25.8±15.6	29.8±21.6	37.2±37.2*	37.7±27.3 *,‡
Alanine transaminase	15.0±8.8	14.3±8.6	14.1±7.1	16.5±10.3	15.8±8.8
HOMA-IR	3.1±1.4	2.5±1.1	3.1±1.4 *,§	2.7±1.0	3.6±1.5 †,‡,**
Insulinogenic index (pmol/mmol)	48.1 (28.8-79.7)	41.8 (24.0-62.2)	50.3 (30.6-81.5)	45.2 (26.9-73.6)	53.8 (32.1-83.0) *

Data are means ± SD, median (inter quartile range) and counts; P values are calculated by One way ANOVA after post-hoc correction with Bonferroni, Kruskall Wallis test (for skewed variables) after post-hoc correction with Dunns and χ^2 test (categorical measures) after correction with Bonferroni. NWTG: Normal waist-line and triglycerides level; iEW: Isolated enlarged waist; iHTG: Isolated hypertriglyceridemia; HTWP: Hyper triglyceridemic waist phenotype; * P< 0.05 vs. NWTG; † P<0.001 vs. NWTG; ‡ P< 0.05 vs. iEW; ¶ P<0.001 vs. iEW; § P<0.05 vs iHT; ** P<0.001 vs. iHTG

4.4.5.3 *Association between insulin resistance and HTWP*

The association between insulin resistance and the categories of abnormalities assessed by multiple linear regression analyses are shown in **Table 4-3**. HTWP was positively associated with insulin resistance after adjustment for an array of potential confounders. iEW showed a significant association with insulin resistance in an unadjusted model, but the association was attenuated when anthropometric and other confounding variables were entered in the equation. iHTG did not show any significant association with HOMA-IR.

Table 4-3: Linear regression analyses showing the association of different abnormalities with insulin resistance.

Variables Baseline	Unadjusted		Model-1		Model-2	
	β (95%CI)	P value	β (95%CI)	P value	β (95%CI)	P value
NWTG	Ref	--	Ref	--	Ref	--
iEW	0.25(0.13-0.36)	<0.0001	0.1 (-0.01- 0.26)	0.052	0.13 (-0.01-0.26)	0.067
iHTG	0.14(-0.02- 0.28)	0.090	0.08 (-0.08-0.22)	0.345	0.04 (-0.12-0.20)	0.620
HTWP	0.38(0.27-0.50)	<0.0001	0.27 (0.14-0.41)	<0.0001	0.23 (0.09-0.37)	0.001

Legend: Dependent variable: Insulin resistance (HOMA-IR – log transformed); data are regression co-efficient (β) and 95% confidence intervals.
Model-1: adjusted for age, BMI, family history of diabetes, hypertension, body fat percentage, smoking and drinking habits.
Model-2: Model-1 further adjusted for 2-hr PG during OGTT and HDL cholesterol.
NWTG: Normal waist-line triglycerides level; iEW: Enlarged waist-line normal triglycerides level; iHTG: Normal waist-line Raised triglycerides level; HTWP: hypertriglyceridemic waist phenotype

4.4.5.4 *Association between HTWP and incident diabetes*

Table 4-4 shows the hazard ratio attributed to the different abnormalities for T2DM variables with and without adjusting for the study groups (intervention vs. standard care), baseline age, BMI, fat%, family history, smoking, drinking alcohol habits (model-1), 2-hr PG, total cholesterol, HDL-C (model-2) and GGT levels (model-3). The HR of the unadjusted model was 1.53 ([95% CI: 1.07–2.20]; P=0.019). The relationship remained significant after adjusting for the above confounding factors (model-3: HR: 1.49 [95%CI: 1.01-2.21]; P=0.047). Addition of insulin resistance to model-3 nullified the significance (model-4; HR: 1.39 [95%CI: 0.92-2.10]; P=0.119). However, addition of β-cell function (as estimated by insulinogenic index, omitting insulin resistance) did not affect the relationship of HTWP with incident diabetes (model-5; HR: 1.59 [95%CI: 1.05-2.23]; P=0.040).

Alternative combinations of cut-offs for waist circumference and triglycerides were explored in relation to incident diabetes and baseline insulin resistance (HOMA IR ≥4.1), but offered no improvement regarding sensitivity and specificity over the chosen cut-off of waist circumference ≥90 cm and triglycerides ≥1.7 mmol/l (results not shown).

Table 4-4: Cox-proportional hazard model for incident diabetes during the 2 year follow-up.

Variables	β (s.e)	HR [95% CI]	P value
Unadjusted	0.43 (0.18)	1.53 [1.07-2.20]	0.019
Model-1	0.38 (0.19)	1.47 [1.02-2.13]	0.041
Model-2	0.41 (0.20)	1.50 [1.01-2.22]	0.043
Model-3	0.40 (0.20)	1.49 [1.01-2.21]	0.047
Model-4	0.35 (0.21)	1.39 [0.92-2.10]	0.119
Model-5	0.41 (0.19)	1.59 [1.05-2.23]	0.040

Legend: Dependent variable: Diabetes Vs non-diabetes
HTWP categories was dichotomized (HTWP vs. others); TG only ≥ 1.7 mmol/l; WC only ≥ 90 cm
Model-1: Adjusted for age, group, BMI, total body fat percentage, family history of diabetes, hypertension, smoking and drinking.
Model-2: model-1 + 2-hr plasma glucose, cholesterol and HDL-C
Model-3: model-2 + GGT
Model-4: model-3 + insulin resistance (HOMA-IR)
Model-5: model-3 + insulinogenic index
β (s.e.): regression co-efficient and standard error
HR [95% CI]: Hazard ratio [95% confidence intervals]

4.4.6 Discussion

The present analyses showed that HTWP is associated with insulin resistance and increased risk of diabetes in Asian Indian men with IGT. Persons with HTWP also showed raised compensatory early phase insulin secretion. Although baseline glycaemia levels was similar in the four subgroups, the presence of HTWP alone conferred an increased risk of diabetes. The findings are in accordance with other studies: a Canadian study of healthy men where HTWP was associated with hyperinsulinemia as well as elevated apolipoprotein B, and small dense low density lipoprotein [251,255], a Chinese-community based prospective study in urban adults which showed an association of HTWP with diabetes [259] and a study in elderly men which showed an increased association of HTWP with glucometabolic risk and decreased

insulin sensitivity [257]. Earlier, our group showed an association of hypertriglyceridemia either in isolation (iHTG) or as HTWP as a strong predictor of atherogenic dyslipidemia and glucose intolerance in a cross-sectional analysis among Asian Indians[258]. The present prospective data suggested a possible etiological association of HTWP with T2DM. This was rendered non-significant by inclusion of IR in the model, suggesting that the association between HTWP and risk of incident T2DM was mediated through insulin resistance.

In this cohort, hypertriglyceridaemia showed a marginal association with incident diabetes, whereas raised waist circumference did not show an association. A strong association of central adiposity with T2DM had been reported earlier by us in a cross-sectional study in Indians [248]. McKeigue PM et al.[134] reported a association between central adiposity and diabetes risk among migrants south Asians in UK . This discordant observation noted in the present study could be due to the fact that the majority of the at-risk participants in our study had enlarged waist circumference at baseline (69.2%), such that enlarged waist circumference per se might have offered less potential for T2DM risk discrimination than in the general population.

In an earlier study of normoglycaemic individuals, we noted a strong association between triglycerides and insulin resistance in the Asian Indian population. Using the ROC procedure, triglycerides concentration of $\geq$ 1.7 mmol/l was found to have a sensitivity of 66.2% and specificity of 62.2% to distinguish insulin resistance (HOMA-IR$\geq$4.1) [249]. The positive association of triglycerides with incident diabetes noted in the present study suggested that it might have a pathogenic role mediated through insulin resistance. However, the effect was enhanced when hypertriglyceridemia co-existed with enlarged waist.

The association of HTWP with incident diabetes was significant even after the adjusting for 2-hr PG and GGT. But, the relationship was attenuated after adjusting for HOMA-IR. Additionally, HTWP had been shown to be associated with increased visceral adiposity in glucose intolerant patients [253]. For a given BMI, Asian Indians have higher waist-hip ratio and increased accumulation of abdominal fat than many other populations[7,248]. The presence of upper body adiposity coupled with hypertriglyceridemia, even in non-obese Asian Indians appears to induce a high degree of insulin resistance[258] even among non obese Asian Indians.

There is no universally accepted, clinically approved numeric expression that defines insulin resistance and β-cell dysfunction. The cut-off for insulin resistance varies among different ethnic groups and, hence the use of these measures requires careful interpretation and expertise which may not be feasible in routine clinical practice [260, 261]. Although the HOMA model provides a robust, clinical and epidemiological tool to assess insulin action, its application may be limited in non-obese participants[260,262]. Plasma insulin is not routinely measured in most clinical laboratories, especially in the developing countries [263]. Hence as an alternative simple approach, clinical measures such as BMI, waist circumference [264], and lipids (HDL-C/TG ratio) [265,266] have been proposed as proxy measures to identify participants with high risk of insulin resistance and compromised β-cell function. However, these measures explain the only a relatively modest amount of the variations in directly measured insulin resistance compared with insulin-based indices. Hence, HTWP offers alternative method to screen for individuals with increased risk of incident diabetes among prediabetic subjects.

The strengths of this analysis are: a) the prospective design of the study; b) the study has been done in Asian Indians who have a high susceptibility for developing diabetes

and c) it shows that even among a group of high-risk individuals with IGT, HTWP could be used to predict incident diabetes. This study also has a few limitations. The study group was comprised only of men and hence, the association of HTWP with incident diabetes in women needs to be studied. Though, imaging data would be ideal for the determination of visceral adiposity, it is generally not feasible for a large epidemiological analysis, due to its high cost and need for specialized technical expertise. The use of HTWP as a predictor of incident diabetes has significant public health importance in implementing preventive studies. It is suggested that presence of HTWP, which can be assessed by routine laboratory measures and can serve as a simple screening tool to identify persons with insulin resistance and therefore at a high risk for diabetes.

4.5 Oral disposition index and incident diabetes

4.5.1 Study rationale

Adaptation of insulin secretion to prevailing insulin sensitivity is a tightly regulated mechanism and a deterioration of each function is found to be the associated with the development of T2DM[25]. As demonstrated by Bergman and associates[267], there is a rectangular hyperbolic relationship between insulin secretion and insulin action and according to this paradigm there is a strict reciprocal relationship between these variables [41,268]. The product of these measures approximates a constant, termed the "disposition index". T2DM occurs only if β-cell function is inadequate to compensate for chronic insulin resistance [268]. Thus, accurate estimation of β-cell function requires determination of both variables because they need to be judged in relationship to each other.

Methods for assessment of insulin action and secretion such as the euglycaemic and hyperglycaemic clamp and intravenous glucose tolerance test minimal modelling and

evaluation of the acute insulin response to intravenous glucose are laborious and expensive procedures and, therefore, are rarely used in the large-scale epidemiological or clinical research setting [267,269]. A variety of surrogate mathematical "paradigm" models derived from fasting (HOMA) or OGTT plasma glucose and insulin concentration measurements have been proposed and validated as alternative measures to evaluate insulin sensitivity and secretion, for example - Matsuda's insulin sensitivity index and the insulinogenic index) [61,67,139,140]. In fact, these measures have been shown to perform reasonably well for discrimination of individuals with differing levels of insulin resistance and β-cell dysfunction. But, the major shortcoming is that these measures were evaluated against the reference methods rather than as antecedents of diabetes. Recently, various versions of the disposition index as derived from OGTT (e.g: 1) insulinogenic index / HOMA-IR; 2) insulinogenic index / fasting insulin and 3) total $AUC_{ins/glu}$* Matsuda's insulin sensitivity index) have been proposed as analogous to the disposition index derived from the frequently sampled intravenous glucose tolerance test (IVGTT, i.e., acute insulin response to glucose, AIRg*insulin sensitivity, Si) and have been shown to have a moderate association with reference methods [78,79].

It is now well established that, a healthy diet with a lower glycaemic load [214,225,270], increased exercise[271] and weight reduction[272] improve insulin sensitivity in individuals with high risk for diabetes. Most studies of the pathogenesis of diabetes and effect of lifestyle intervention on the improvement of insulin sensitivity and β-cell function among high-risk individuals have been conducted in white populations [214,225,270-272]; very few studies have addressed the pathogenic mechanisms in Asian Indians [30,61]. Asian Indians have a strong predisposition for T2DM and have several peculiar pathophysiological features including a young age of onset of diabetes, a relatively

lower BMI, with high rates of insulin resistance, and lower thresholds for the risk factors for diabetes [273] compared with Caucasians.

The objective of this analysis is to assess the predictive power of baseline disposition index derived from the OGTT in relation to the subsequent incidence of T2DM and to study the association of lifestyle parameters (dietary compliance and physical activity) with changes in oral disposition index. We hypothesize that, higher β-cell compensation at baseline, and improvement of the same during intervention, will be associated with decreased incidence of diabetes. We also hypothesizes that any improvement in the oral disposition index is mediated through improved dietary compliance in this study.

4.5.2 Methods

The description of the cohort was already explained in detail in **Chapter-2 and 3**. For this present analysis, we combined both the groups as a single cohort to unravel the association of oral disposition index with incident diabetes.

4.5.3 Statistical analysis

A relationship between insulin sensitivity and insulin secretion is thought to be a "*rectangular hyperbola*" wherein the product of the two measures approximates a constant. The validity of oral disposition index was assessed by demonstrating a hyperbolic relationship between the measure of insulin secretion and insulin sensitivity derived from the OGTT. Regression analysis was applied to different combinations of insulin secretion and insulin sensitivity index to determine the regression coefficient β for the following model: ln(insulin secretion) = constant + β× ln(insulin sensitivity). In this context, the product of OGTT-based surrogate insulin measures obeys a hyperbolic relationship if the following criteria are satisfied: (i) regression coefficient

(β) is approximately equal to −1 and if the 95% confidence interval (CI) of β includes -1 and excludes 0[82,274].

Results of the OGTT were used to designate a participant's glucose tolerance status as: regressors (IGT to NGT), unchangers (IGT to IGT) and progressors (IGT to T2DM). Normally distributed variables were compared across glycaemic categories using one-way ANOVA after post-hoc corrections according to Bonferroni. For non-parametric variables Kruskal- Wallis test with Dunn's post-hoc corrections was used. Changes from baseline to follow-up were compared between groups by t test when variables were normally distributed and by Wilcoxon's test for variables non-normally distributed. The power of oral disposition index and other surrogate insulin measures to predict a progression to diabetes was assessed with a ROC curve[216,217]. All analyses were performed using SPSS software (IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp).

4.5.4 Results

The baseline characteristics of participants stratified according to their glycaemic outcome at the end of the study were shown previously (**Table 4-1**).

4.5.4.1 *Demonstration of hyperbolic relationship*

Using OGTT data, the two measures of insulin secretion (1) $AUC_{ins/glu}$ from 0 to 2-hr; 2), insulinogenic index) and three measures of insulin sensitivity (1) 1/HOMA-IR; 2) 1/fasting insulin; 3) Matsuda's insulin sensitivity index were tested for the existence of a hyperbolic relationship. The following combinations of composite β-cell function were calculated from the OGTT: a) insulinogenic index / HOMA-IR; b) insulinogenic index /fasting insulin; c) insulinogenic index * Matsuda's insulin sensitivity index; d)

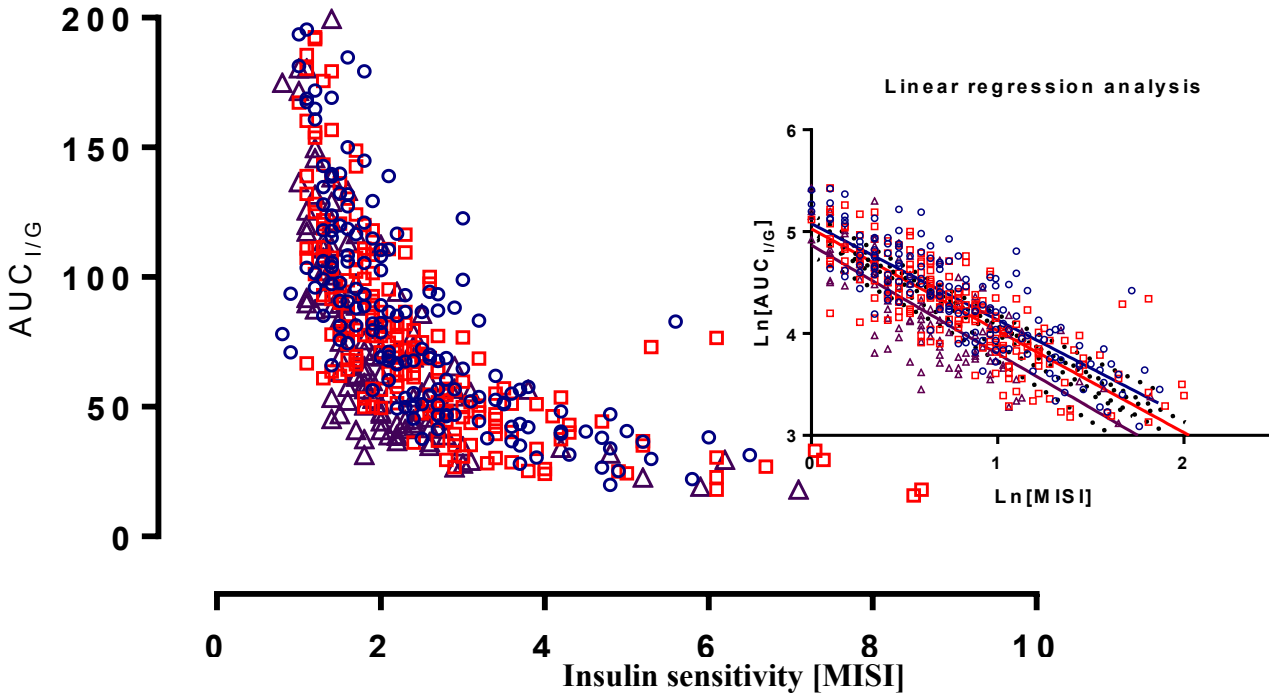
$AUC_{ins/glu}$ from 0 to 2-hr/ HOMA-IR and e) $AUC_{ins/glu}$from 0 to 120 min * Matsuda's insulin sensitivity index (ISSI-2) [79].

As shown in **Table 4-5**, the only pairing that satisfied both of the hyperbolic criteria was that of total $AUC_{ins/glu}$ and Matsuda's insulin sensitivity index (β: -0.954 [95% CI: -1.015 to -0.893]). Importantly, the pairing of total $AUC_{ins/glu}$ and Matsuda's insulin sensitivity index yielded distinct hyperbolae for each glucose tolerance group (NGT: β: -0.946 [95% CI: -1.048 to -0.842]; IGT: β: -1.000 [95% CI: -1.085 to -0.915]; T2DM: β: -1.063 [95% CI: -1.202 to -0.923]), consistent with the existence of a hyperbolic relationship (**Figure 4-4**). The mean product of total $AUC_{ins/glu}$ and Matsuda's insulin sensitivity index progressively decreased from NGT to IGT to T2DM consistent with the notion of declining of β-cell function across these groups (IGT to NGT: 175.5 ± 56.1; IGT to IGT: 159.3 ± 49.3; IGT to T2DM: 130.5 ± 52.5); P <0.0001). The product of total $AUC_{ins/glu}$ and Matsuda's insulin sensitivity index was therefore taken as the measure of oral disposition index in this cohort.

4.5.4.2 *Factors associated with improvement in oral disposition index*

Multiple linear regression analysis adjusted for age, family history of diabetes, baseline and change in BMI, dietary energy intake and physical activity demonstrated that weight reduction (β: -11.7 (95%CI: -8.1 to -15.2)) and decreased energy intake (β: -4.0 (-2.8 to -5.2)) from baseline were independently associated with improvement in oral disposition index.

Figure 4-4: Demonstration of hyperbolic relationship



Legend: Estimated regression coefficient (β) and 95% CI for β, for combinations of insulin secretion, and sensitivity measures regressed using the following model: log(secretion measure) = constant + β × log(sensitivity measure); Overall: Y= -0.954*X+4.982 O: NGT: Y= -0.946*X+5.079; □: IGT: Y= -1.000*X+5.028; Δ: DM: Y= -1.063*X+4.868

Table 4-5: Demonstration of hyperbolic relationship between surrogate measures of insulin secretion and sensitivity

Secretion	Sensitivity	β	95% CI
Insulinogenic index	1/HOMA-IR	-0.496	-0.620 to -0.371
Insulinogenic index	1/Fasting insulin	-0.621	-0.745 to -0.496
Insulinogenic index	Matsuda's insulin sensitivity index	-0.865	-0.977 to -0.754
$AUC_{ins/glu}$	1/HOMA-IR	-0.584	-0.672 to -0.496
$AUC_{ins/glu}$	1/Fasting insulin	-0.689	-0.774 to -0.603
$AUC_{ins/glu}$	Matsuda's insulin sensitivity index	-0.954	-1.015 to -0.893

4.5.4.3 *Change in oral disposition index levels according to glycaemic status at the end of the study*

Overall, transition from IGT to NGT was associated with an improvement of insulin sensitivity and oral disposition index, and a reduction in insulin secretion (**Figure 4-5**, Panels A-C; **Table 4-6**). In individuals who underwent the transition IGT to T2DM, there were reductions in insulin sensitivity (-4.8%) and oral disposition index (-34.6%) and a greater reduction in insulin secretion (-28.1%) than in those undergoing the IGT to NGT transition. In individuals who remained IGT during the study, there was an intermediate increase in insulin sensitivity and a decrease insulin secretion and no change in oral disposition index.

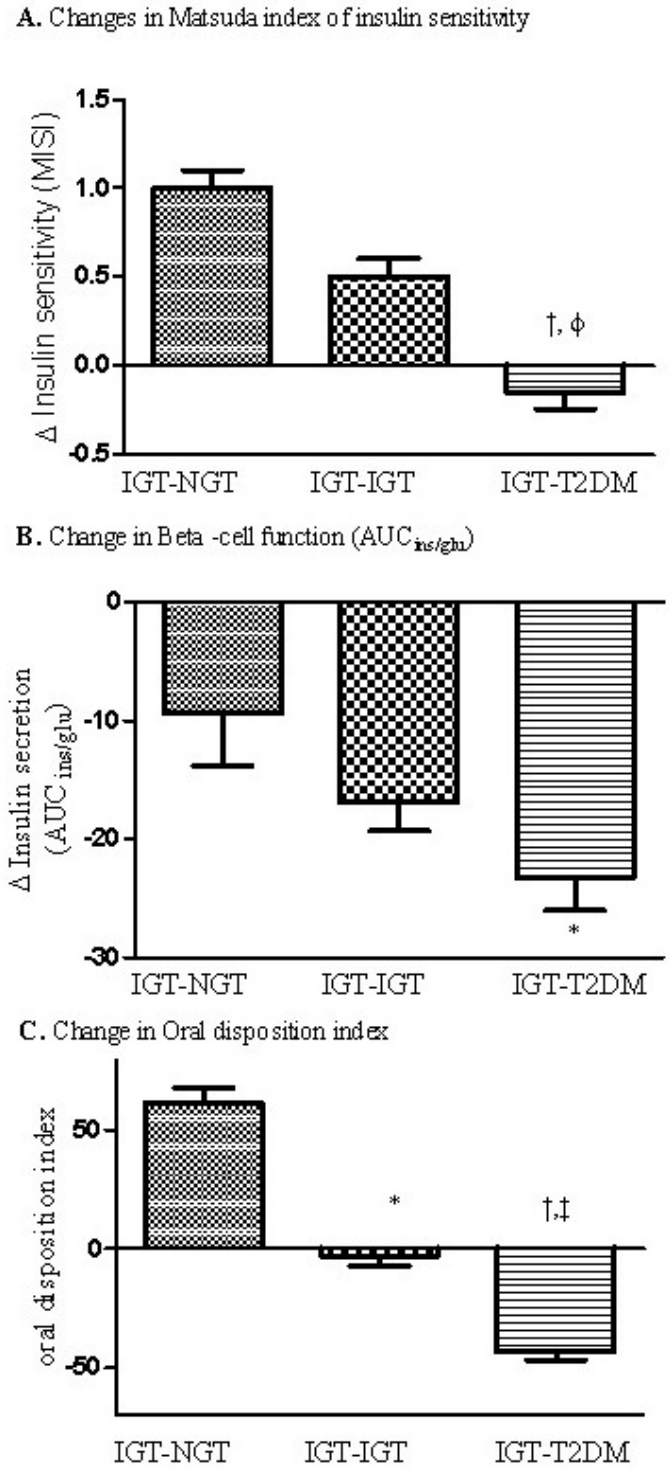
To illustrate the changes in oral disposition index associated with the three different transitions during the course of the study, insulin secretion was plotted against sensitivity in each of the three groups at baseline and then on completion of the study; in this way, six hyperbolic curves were generated and the point on each curve located by the mean insulin secretion and mean insulin sensitivity for each curve represented the oral disposition index (**Figure 4-6**). In the group that underwent the IGT to NGT transition, there was a rightward shift from the baseline towards higher insulin sensitivity and improved oral disposition index, whereas in the group undergoing the IGT to T2DM transition there was a shift toward the origin of the plot, with lower insulin secretion and sensitivity and lower oral disposition index. In the IGT to IGT group there was little apparent change.

Table 4-6: Baseline and final levels of insulin sensitivity, insulin secretion and oral disposition index according to glucose tolerance categories

	Baseline	**Follow-up**	**% change**	**P value**
IGT to NGT				
Insulin sensitivity index	2.4±1.4	3.4±1.3	41.7	<0.0001
$AUC_{ins/glu}$	81.2 (51.5-110.7)	68.6 (52.8-92.2)	-15.5	0.003
Oral disposition index	168.8 (136.3-203.8)	217.5 (179.3-281.0)	28.9	<0.0001
IGT to IGT				
Insulin sensitivity index	2.6±1.5	3.0±1.7	15.4	<0.0001
$AUC_{ins/glu}$	71.2 (48.3-99.1)	54.8 (38.9-74.7)	-23.0	<0.0001
Oral disposition index	154.8 (125.3-184.1)	146.1 (113.9-180.3)	-5.6	0.115
IGT to T2DM				
Insulin sensitivity index	2.1±1.0	2.0±0.8	-4.8	<0.0001
$AUC_{ins/glu}$	62.6 (43.9-94.3)	45.0 (34.3-60.1)	-28.1	<0.0001
Oral disposition index	124.1 (101.3-155.8)	81.2 (69.6-100.8)	-34.6	<0.0001

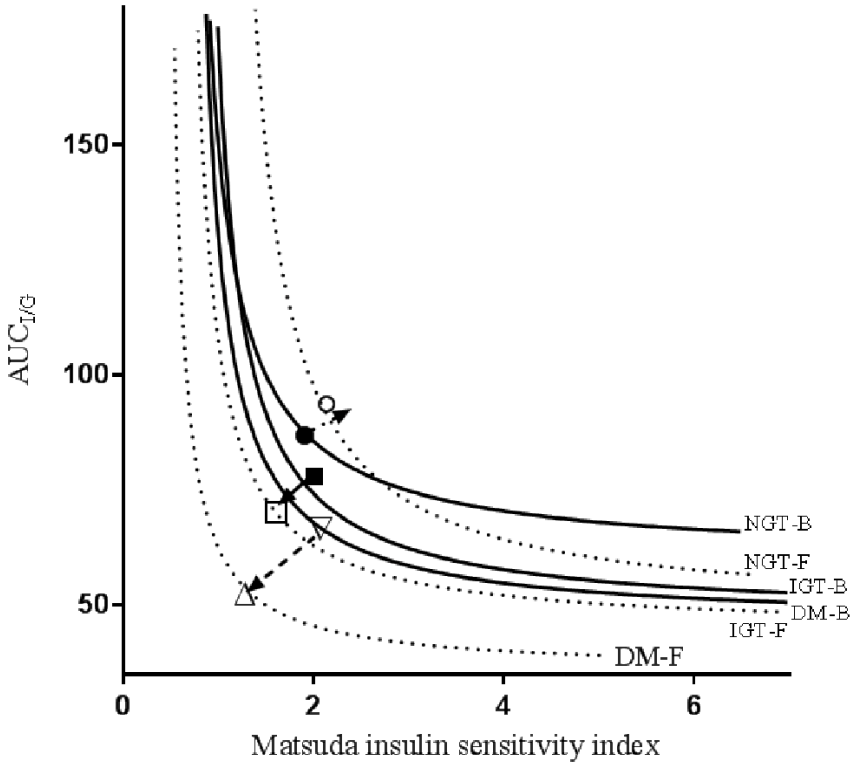
Legend: Data are mean ± SD for normally distributed variables and median (inter quartile range) for skewed variables; IGT: impaired glucose tolerance; NGT: normal glucose tolerance; DM: diabetes; $AUC_{ins/glu}$: ratio of total area-under-curve between insulin and glucose. % change is calculated by (final values –baseline values / baseline values)*100.

Figure 4-5: Changes in insulin measures throughout the study. Individuals were all IGT at baseline and groups were distinguished by glycaemic status at the end of the study



Legend: Changes in Matsuda's insulin index (A), AUCins/glu ratio (B) and oral disposition index (C) throughout the study. Individuals were all IGT at baseline and groups were distinguished by glycaemic status at the end of the study. Change represents the difference between the absolute value at the study end minus baseline. * P< 0.05 vs. NGT; † P<0.001 vs. NGT; ‡ P< 0.05 vs. IGT; ¶ P<0.001 vs. IGT.

Figure 4-6: Change in oral disposition index in relation to the final glycaemic outcomes



Legend: The baseline and final year oral disposition index are plotted for individuals with NGT, IGT and DM relative to the hyperbolic curves; individuals deteriorated to diabetes had decreased β cell function at baseline. Those who regressed to normoglycaemia showed an improvement in β cell function at the end of follow-up. Symbols represent the mean values. ●: NGT baselibe; ○ : NGT Final; ■: IGT baseline; □: IGT final; ▽: T2DM baseline; Δ: T2DM final;

4.5.4.4 *Discussion*

In the present analyses we have demonstrated a hyperbolic relationship between OGTT-based total $AUC_{ins/glu}$ and the Matsuda's insulin sensitivity index in Asian Indian men with prediabetes. Importantly, IGT individuals with better β-cell compensation at baseline were the least likely to develop diabetes. These observations were consistent with the results of other studies: a prospective study in Hispanic women with gestational diabetes [75], the ACT NOW study [77] and an observational study in Japanese-American individuals which showed a strong association of oral disposition index with incident diabetes over 10 years duration [82]. A recent cross sectional analyses in 1264 individuals conducted in Asian Indians had also found early reductions in β-cell function as a primary etiological factor for the development of diabetes [275].

In this cohort, the combination of total $AUC_{ins/glu}$ and the Matsuda insulin sensitivity index yielded distinct hyperbolae for different degrees of glucose tolerance. Furthermore, these hyperbolae exhibit a shift towards the origin as glucose tolerance worsens, in a manner analogous to the disposition index curves measured by euglycaemic and IVGTT methods [53]. This shift in hyperbola position is a hallmark of T2DM pathophysiology and is considered as one of the earliest indicators of β-cell dysfunction[53].

Although, insulin resistance is a core pathophysiologic abnormality in the progression from NGT to IGT to diabetes, overt diabetes only occurs if β-cells fail to compensate for compounding insulin resistance. As reported by Petersen et al [276], β-cell compensation for a given insulin resistance is around 60% lower in healthy Asian Indians compared with White populations matched for age, sex, BMI and lifestyle

factors. These findings suggest the possibility of ethnic differences in the optimal relationship between insulin sensitivity and insulin response[54]. Declining β-cell compensation may be the primary aetiological precursor for glycaemic deteriorations from NGT to diabetes[277].

In this cohort, decreased total dietary energy consumption from baseline and minimal weight reduction was associated with significantly improved β-cell function. Given the relative abundance of energy supply in much of the westernized world, the compensatory ability of the β-cell is the single physiological process that protects well-fed individuals from the scourge of diabetes. While individuals living in developed nations have long been able to muster adequate food supplies, populations recently exposed to energy dense food have an inherent inability to adapt metabolically to the high calorie diet compared with the Caucasians [278].

In summary, based on these findings, we infer that an OGTT derived measure of disposition index may be an alternate surrogate measure to identify high risk individuals. Importantly, improving insulin sensitivity by caloric restriction and minimal weight reduction may have beneficial effects on β-cell function and prevent diabetes for relatively long periods of time in genetically susceptible individuals.

4.6 Comparison of classical risk factors

Table 4-7 shows the ability of the baseline glycaemic measures, GGT, HTWP, insulin sensitivity and β-cell function to predict incident T2DM, as explored in this analysis, and their corresponding optimal cut-off points in predicting incident diabetes. As expected baseline FPG ≥ 5.6mmol/l (AUC: 0.615 [95%CI: 0.558-0.672]), 2hr PG ≥ 8.6 mmol/l (AUC: 0.670 [95%CI: 0.614-0.725]) and HbA1c ≥ 6.1% (AUC: 0.674 [95%CI: 0.616-0.732)]) predicted incident diabetes in this cohort.

Table 4-7: Area under the receiver operating characteristics and predictabilities of glycaemic, non-glycaemic and surrogate insulin indices for progression of diabetes [a]

Indices	AUC ROC (95% CI)	P value[b]	Sensitivity % (95% CI)	Specificity % (95% CI)	Positive likelihood ratio
Glycaemic measures					
2hr PG	0.675 (0.614-0.725)	<0.0001	58.5 (49.3-67.3)	67.5(62.6-72.1)	1.8
FPG	0.620 (0.558-0.672)	<0.0001	78.1(69.7-85.0)	40.9(36.0-45.9)	1.3
HbA1c	0.677 (0.634-0.717)	<0.0001	56.9(47.7-65.8)	71.1(66.3-75.5)	2.0
Non-Glycaemic markers					
GGT	0.637 (0.593-0.679)	<0.0001	71.1(62.1-79.0)	52.9(47.7-57.9)	1.5
HTWP [c]		0.016	41.0 (33.0-51.0)	71.0 (66.0-75.0)	1.4
Combination of GGT and glycaemic measures					
FPG+GGT	0.668 (0.613-0.722)	<0.0001	59.5 (50.2-68.3)	64.8(59.8-69.6)	1.70
2hrPG+GGT	0.705 (0.653-0.757)	<0.0001	74.4(65.6-81.9)	58.1(53.0-63.1)	1.77
HbA1c+GGT	0.702 (0.648-0.755)	<0.0001	59.5(50.2-68.3)	71.6(66.8-76.1)	2.1
Insulin surrogate measures					
Oral disposition index	0.717 (0.675-0.756)	<0.0001	70.0 (61.0-78.0)	64.1 (59.0-68.9)	1.95
HOMA-IR	0.642 (0.598-0.685)	<0.0001	59.2 (49.8-68.0)	62.7 (57.6-67.6)	1.58
1/Fasting insulin	0.615 (0.570-0.658)	0.001	73.3 (64.5-81.0)	43.7 (38.6-48.9)	1.30
MISI	0.598 (0.554-0.642)	0.0005	61.7 (52.4-70.4)	53.9 (48.7-59.0)	1.34
Insulinogenic index	0.599 (0.554-0.642)	0.0013	45.0 (35.9-54.3)	72.5 (67.7-77.0)	1.64
$AUC_{ins/glu}$	0.554 (0.509-0.599)	0.0728	60.0 (50.7-68.8)	50.4 (45.2-55.6)	1.19
HOMA-β	0.530 (0.485-0.575)	0.3104	85.8 (78.3-91.5)	23.7 (19.5-28.4)	1.13

Legend: a: Diabetes was diagnosed based on WHO recommendations; b: Significance level P (Area=0.5); c: P value computed by categorical contingency analysis; The units of each optimal cut-off point was shown in respective tables. 2hr PG: 2hr plasma glucose; FPG: fasting plasma glucose; GGT: gamma glutamyl transferase, HTWP: hypertriglyceridemic waist phenotype; HOMA-IR: homeostatsis model assessment of insulin resistance; MISI: Matsuda's insulin sensitivity index.

Among the glycaemic measures, baseline levels of HbA1c showed the highest predictive value for detecting diabetes. As already described, baseline GGT levels of ≥ 26.4 UL^{-1} predicted incident diabetes over a 2 year period (AUC: 0.637 [95%CI: 0.581-0.692]; P< 0.0001; sensitivity: 61.2%; specificity: 60.9%) in this cohort. In separate analyses, we have studied the predictive utility of GGT when combined with the glycaemic measures (**Table 4-7**). The results showed that the model comprising of FPG and GGT (AUC: 0.668 [95%CI: 0.613-0.722]; P<0.0001) was equally effective in identifying individuals with risk of diabetes as compared to the 2-hr PG and HbA1c. Furthermore, the addition of GGT to glycaemic measures improved the sensitivity for detecting diabetes. The association of HTWP with incident diabetes was found to be modest (specificity: 71.6 (66.8-76.1); sensitivity: 41.0 (33.0-51.0)).

Among the surrogate insulin measures, the area under the ROC curve was highest for the oral disposition index (AUC: 0.717 [95%CI: 0.664-0.770]). The optimal cut-off value for predicting incident diabetes for the oral disposition index was ≤ 144.7 (sensitivity: 70.0 (95%CI: 61.0-78.0); specificity: 64.1 (95%CI: 59.0-68.9)). Other surrogate insulin measures, HOMA-IR (AUC: 0.642 [950.598-0.685]), 1/fasting insulin (AUC: 0.615 [95%CI: 0.570-0.658]), Matsuda's insulin sensitivity index (AUC: 0.598 [95%CI: 0.554-0.642]) and insulinogenic index (AUC: 0.599 [95%CI: 0.554-0.642]) also showed significant predictability. In this cohort, total $AUC_{ins/glu}$ did not show an association with T2DM. This discordant observation noted in the present study could be due to the fact that IGT itself is a compromised β-cell responsive state[279], so total $AUC_{ins/glu}$ per se may have offered less potential for T2DM risk discrimination. Furthermore, $AUC_{ins/glu}$ ratio may not be a good surrogate index for predicting diabetes[280].

4.7 Classical risk factors as a predictor of diabetes - Final thoughts

In this cohort, oral disposition index was a better predictor of incident diabetes compared with other OGTT-based insulin measures such as the combination of HOMA-IR and insulinogenic index. Previously, our group showed independent associations of both insulin resistance and impaired early insulin secretion in the development of diabetes[61]. The oral disposition index as a composite measure may be a better index than either insulin secretory measure or sensitivity alone. There seems to be an ethnic disparity in the interaction between insulin action and secretion in Asian Indians. For a given BMI and waist circumference, Asian Indians usually have a poorer β-cell reserve and decreased insulin sensitivity compared with Caucasians[281]. As reported by Petersen et al [276], β-cell compensation for a given insulin resistance is around 60% lower in healthy Asian Indians compared with White populations matched for age, sex, BMI and lifestyle factors. These findings suggest the possibility of genetic determinants that cause early β-cell failure in the presence of chronic insulin resistance.

Not surprisingly, glycaemic measures were predictive of incident diabetes. In this cohort, baseline GGT levels showed a modest association with diabetes, possibly suggesting its role in the pathogenesis of diabetes. In this analysis, additive values of combining GGT and glycaemic measures in prediction of incident diabetes were also studied. The diagnostic power was moderately improved when GGT was added to FPG or HbA1c. Therefore, we propose that combination of measures of glycaemia (FPG and HbA1c) and GGT may be an alternate and sensitive tool to identify individuals at risk. But, it should be emphasize that neither HbA1c nor GGT levels provide information about oral disposition in this cohort.

Declining β-cell compensation may be the primary aetiological precursor for glycaemic deterioration from NGT to diabetes. However, this measure of β-cell function requires performance of the OGTT with determination of the plasma insulin and glucose concentrations. The existing glucose-based diagnostic tests have a few performance limitations[282]. The OGTT is the reference test for the diagnosis of diabetes according to World Health Organization (WHO)[85]. But it is time-consuming, unpleasant for the patients and not used routinely in large epidemiological studies. Though, FPG is easy to perform it has poor specificity for identifying high risk individuals, especially in Asian populations. Recently, the American Diabetes Association (ADA) and the WHO recommended HbA1c as a surrogate measure of chronic hyperglycaemia over 8-12 weeks for diagnosis of diabetes[83,87]. In our cohort, the combination of HbA1c and GGT was found to be associated with diabetes. In fact, this composite measure was as predictive as the disposition index for this purpose. In an epidemiological setting, the use of the OGTT may be limited. Therefore, we propose that a simple clinical model comprising of HbA1c and GGT, both of which are routinely available, could provide an alternate screening tool to identify individuals with high risk of developing diabetes. These findings could have important public health implications.

5 ASSOCIATION OF NOVEL BIOMARKERS AND DIABETES

5.1 Background

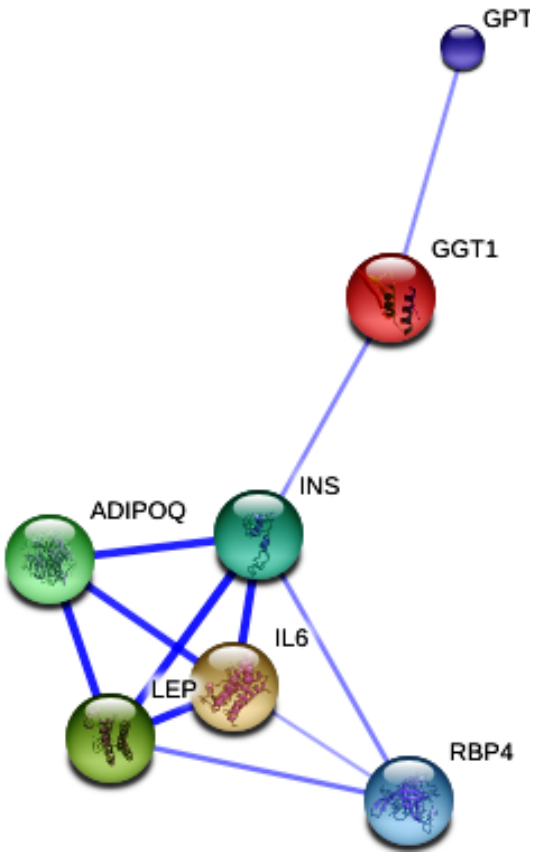
In addition to the "classical" biomarkers derived from glycaemic measures, surrogate insulin measures, hepatic enzymes and abnormal lipid profile, there is a broad range of measurements of specific plasma proteins that may provide valuable information concerning type 2 diabetes (T2DM) risk. Candidates for a "novel panel" of biomarkers were selected from searches of the PubMed database using search terms associated with mechanisms known to be important in the development of diabetes such as carbohydrate and lipid metabolism, insulin resistance, β- cell dysfunction, inflammation and oxidative stress. Over 1000 abstracts were reviewed; from this rigorous literature research I shortlisted 7 biomarkers for this pilot project.

5.2 Justification of proposed biomarkers

5.2.1 Justification: systems biology approach:

In order to explore the interaction of these selected biomarkers and illustrate their functional complexity, selection of this panel was informed by the systems biology bioinformatics tool STRING (Search Tool for the Retrieval of Interacting Genes) [283,284]. This tool is an exploratory database designed with the goal to assemble, evaluate and disseminate protein-protein association information in a user-friendly and comprehensive manner. The tool has been employed here to distinguish networks of association between potential biomarkers and illustrate the strengths of association between them. In the database, the associations between proteins are derived from: 1) experimental evidence; 2) manually curated database evidence (from, for example, the Kyoto Encyclopaedia of Genes and Genomes (KEGG) database)[285]; 3) text mining

evidence (PubMed)[286]; and 4) genomic context analysis (co-expression and neighbourhood analysis)[287]. These associations are then quantitatively expressed by a probabilistic confidence score ranging from 0 to 1. This is computed under the assumption of independence for the various sources, in a naïve Bayesian fashion[284]. **Figure 5-1** shows the STRING-generated network view of the proposed biomarkers under investigation.



Variable-1	Variable-2	Score*
ADIPOQ	LEP	0.954
ADIPOQ	INS	0.923
ADIPOQ	IL-6	0.878
RBP4	IL-6	0.432
RBP4	LEP	0.613
LEP	INS	0.980
IL-6	INS	0.968
GGT1	INS	0.542
GGT1	GPT	0.508

Figure 5-1: Graphical representation of association / functional interaction of proposed biomarkers

Legend: STRING generated network of proposed biomarker (http://string-db.org/newstring_cgi/show_input_page.pl). Stronger associations are represented by thicker lines. From this network view we can affirm that these proteins are associated with each other functionally. Cross-talk between these functional proteins might then mean that derangement of function in one protein could affect proteins involved in distal pathways and promote pathogenesis of T2DM. ADIPOQ: adiponectin, LEP: leptin, INS: insulin, RBP4: retinol binding protein-4, IL-6: interleukin-6, GGT: gamma-glutamyl transferase, GPT: Alanine transaminase. *: Confidence scores are derived from the addition of: 1) experimental evidence; 2) manually curated database evidence; 3) text mining evidence (PubMed); and 4) genomic context analysis (co-expression and neighbourhood analysis); confidence range (low confidence: scores<0.4; medium: 0.4 to 0.7; high:>0.7)

5.2.2 Justification - mechanistic approach:

Mechanistic justifications for this network can be found in well-established biochemical and physiological connections [288]. For instance, abnormal levels of chemokines released by the expanding adipose tissue during obesity and insulin resistance impair insulin action by altering the secretion of cytokines, specifically of leptin and adiponectin [289], and also leads to inflammatory conditions, with increased inflammatory cytokines such as interleukin-6 (IL-6). Such cytokines, in turn, increase insulin resistance in adipose tissue, skeletal muscle and liver. This contemporaneous effect contributes to an unfavourable shift in the pro-inflammatory / anti- inflammatory balance. Leptin, apart from its role in energy expenditure and inflammation, also indirectly reduces the levels of the anti-oxidant enzyme Paraoxonase-1(PON1) [290] in the circulation and could, therefore promote oxidation of LDL particles. Usually, Asian Indians have elevated levels of free fatty acids in plasma [291] , which can result in accumulation of ectopic fat, non alcoholic fatty liver disease and increased levels of liver enzymes in circulation. Hepatocytes and adipocytes also secrete retinol binding protein 4 (RBP4), the specific transport protein for retinol (vitamin A)[292-295]. Several studies have demonstrated that increased levels of RBP4 result in insulin resistance in peripheral tissues such as liver and skeletal muscle by an interaction with the insulin-sensitive GLUT4 glucose transporter[296-298]. A normal level of vitamin D itself is essential for the release of insulin from pancreatic β- cells and decreased levels have been related to development of β- cell dysfunction [299]. However, these reports are not uniformly confirmed in other studies[300].

Thus, the rationale of using one biomarker to predict T2DM is uncertain, when the risk of progression to T2DM actually varies with a range of biomarkers affected in various

pathways. This justifies study of the co-association of multiple biomarkers in the development of T2DM.

5.3 Clinical relevance of proposed biomarkers

The clinical relevance of each biomarker is explained in detail in the subsequent section.

5.3.1 Adiponectin

Epidemiological evidence:

- In Asian Indian individuals (IDPP-1) with lower BMI, lower adiponectin level at baseline was a strong predictor of future diabetes (OR 0.87 [0.79-0.95]) [301].
- In the Insulin Resistance and Atherosclerosis study (IRAS) family study in 1,906 Hispanic, American Africans participants, baseline adiponectin level was associated with incident diabetes (0.54 [CI 0.38–0.76]). The association remained significant after adjustment in individual models for BMI, HOMA-IR and visceral adipose tissue [302].
- The DPP study showed that baseline adiponectin and improvement in adiponectin level was associated with reduced incidence of diabetes [303].
- A recent meta-analysis of 13 prospective studies with a total of 14, 598 participants and 2,623 incident cases of T2DM provided unequivocal evidence that higher adiponectin levels are associated with a reduced risk of diabetes (HR 0.72 [0.67-0.78]) [304].

Adiponectin – In brief:

Molecular weight: 30 KDa

Synthesis: Exclusively synthesized from adipocytes.

Chromosome location: 3q27

Structure: Collagen-like domain; structural similarity with Complement C1Q

Plasma half-life: 2.5hr

Clearance: Mainly in liver

Function:

- Normal metabolic homeostasis
- Stimulate beta-oxidation in muscle and increase insulin sensitivity in liver.
- Anti-inflammatory activity

Remarks:

Unlike other adipokines "adiponectin" levels decrease with increased fat mass

5.3.2 Interleukin-6

Epidemiological evidence:

- In the British Regional Heart Study (BRHS), IL-6 predicts incident diabetes (HR: 2.12 [1.18-3.81]), after adjustment for BMI, lifestyle factors and insulin resistance [305].

- In the Multi-Ethnic Study of Atherosclerosis (MESA), involving 5,571 participants, IL-6 was associated with incident diabetes (HR: 1.5 [95%CI: 1.1–2.2]) [306] after adjustment for age, sex, race, lifestyle factors, HOMA-IR and BMI.

- A recent meta-analysis of 10 prospective studies with a total of 19, 709 participants and 4,480 incident cases of T2DM demonstrated a significant dose-response relationship of IL-6 with incident diabetes (relative risk [RR] 1.31 [95%CI 1.17–1.46]) [307].

Interleukin 6 – In brief:

Molecular weight:~20-30 KDa

Synthesis: activated leukocytes, endothelial cells, and adipocytes.

Chromosome location:7p21

Structure: helical glycoprotein

Plasma half-life:<6.0 hr

Clearance: Mainly in Kidney

Function:

- Involved in acute phase response
- Role in bone metabolism, reproduction, arthritis, neoplasia, and aging.
- It is also associated with lifestyle diseases such as diabetes, cancer and cardiovascular diseases.

5.3.3 Leptin

Epidemiological evidence:

- In the British Regional Health Study, leptin (top Vs bottom tertile) was associated with a relative risk of diabetes (HR: 1.91 [95%CI: 0.97-3.76]), after adjustment for potential confounders, but further adjustment for insulin resistance abolished the association [305].
- In Japanese Americans, greater baseline leptin levels were associated with an increased risk of developing diabetes (HR: 1.8 [95%CI: 1.02-3.17]) in men but not in women [308]. A similar finding was observed in Mauritian men but not in women [309] and in the PROspective Study of Pravastatin in the Elderly at Risk trial (PROSPER) study [310].
- In a multi ethnic, Atherosclerosis Risk In Community study (ARIC), was significantly associated with a 40% lower risk of subsequent diabetes [311] after adjustments for potential covariates.

Leptin – In brief:

Molecular weight: 16 KDa

Synthesis: Mainly in adipocytes and in low levels by the gastric fundic epithelium, intestine, skeletal muscle, and brain

Chromosome location: 7q31.3

Plasma half-life: 25 min

Clearance: Mainly in Kidney

Function:

- Food intake and energy metabolism
- Body weight regulation, puberty and reproduction
- Immune regulation

Remarks: Exhibits diurnal rhythm and levels varies between sex.

5.3.4 Retinol Binding Protein-4 (RBP4)

Epidemiological evidence:

- Cross-sectional study from South Korea demonstrated that RBP4 concentrations were raised in IGT and in T2DM [312].
- Circulating RBP4 is associated with hepatic insulin resistance [298], and its levels are elevated in obese individuals [298,313] and in association with cardiovascular risk factors such as raised lipid levels and insulin resistance [314].
- In the Co-operative Health Research in the Region of Augsburg (KORA) F4 study, RBP-4 (top Vs bottom quartile) level was associated with prediabetes independent of known metabolic and lifestyle variables (OR: 1.63[1.17-2.27] [315].
- In the ARIC study, there was a borderline association between serum RBP4 levels (top Vs bottom tertile) and diabetes (HR: 1.68; [95%CI:1.00-2.82])[316] after adjusting for demographic, metabolic and clinical variables in women.

RBP4 – In brief:

Molecular weight: ~ 21 KDa

Expression: Liver and mature adipocytes.

Chromosome location: 10q23–q24

Structure: Lipocalin family

Plasma half-life: 12hr

Clearance: Renal excretion

Function:

- Vitamin A transport protein.
- Prevents the excretion of transthyretin by kidney
- Increased RBP4 levels increases gluconeogenesis in liver and decreases insulin sensitivity in muscle.

Remarks:

Adipokine associated with obesity and insulin resistance

5.4 Research methods:

5.4.1 Study participants:

The complete study design, eligibility criteria, recruitment of participants, and methods have been described elsewhere (**Chapter-2** and **3**). **Figure-5-3** shows the pictorial representation of sample stratification for the present study. At the end of the study, of the 517 (96.3%) responders:

- 170 (34.6%) had reverted to normoglycaemia (non-progressor).
- 224 (43.3%) had remained IGT (non-progressor).
- 123 (23.8%) had progressed to diabetes (Progressors).

For this sub study, we had selected 71 newly detected diabetes cases (i.e. IGT to T2DM) and 76 randomly selected controls (43 IGT to NGT and 33 IGT to IGT).

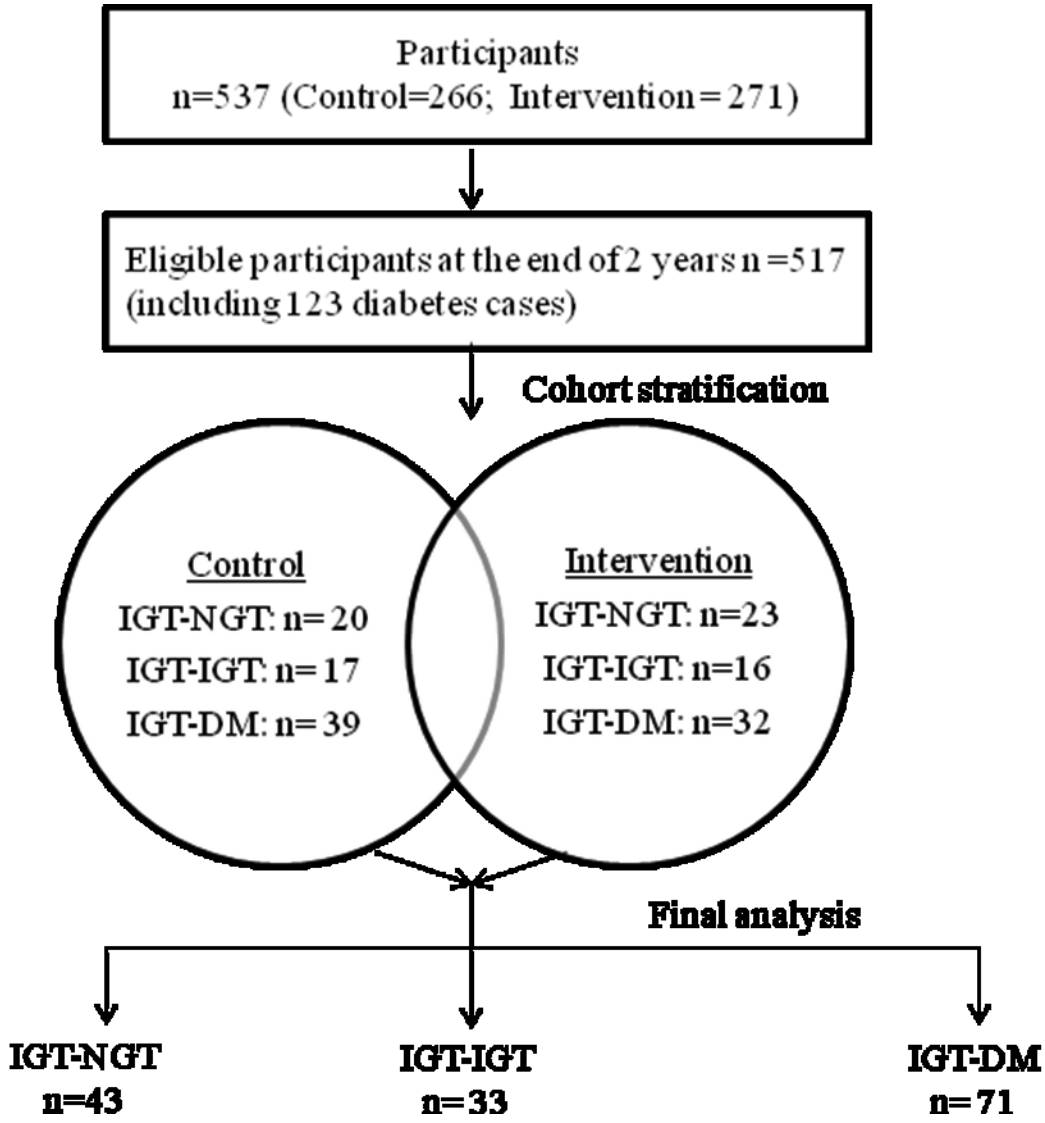


Figure 5-2: Selection of samples for biomarker analysis

Legend: At the end of the study, 497 participants were responded for the 2nd year follow-up, among them 71 cases (diabetes) and 76 control (NGT: 43; IGT: 33) were chosen for the analysis.

5.4.2 Measurements:

Routine anthropometric and clinical measurements were described in **Chapter-2**.

5.4.3 Analytical procedures:

Measurements of biomarkers with typical standard curves shown in **Figure 5.3**. Plasma leptin were measured by were determined using sandwich enzymelinked immunosorbent assay (ELISA) (dbc diagnostics, canada). The intra- and interassay coefficients of variation (CVs) were <5.5% and <7% respectively, over the sample concentration range 1-100 ng/ml. The detection limit of the assay was 1 ng/ml. Plasma adiponectin concentrations were determined using solid phase ELISA (Organium, Finland.). The intra- and interassay CVs were <10% and <12%, respectively. The detection limit was <0.185 ng/ml. IL-6 was assayed using a sandwich ELISA (eBiosciences). The intra- and interassay CVs were each <10%. Plasma RBP4 were measured by competitive enzyme immune assay (EIA) (RayBiotech, Norcross). The intra- and interassay CVs were <10% and <15% respectively. All the biomarkers were estimated in a freshly freeze-thawed stored plasma fasting samples.

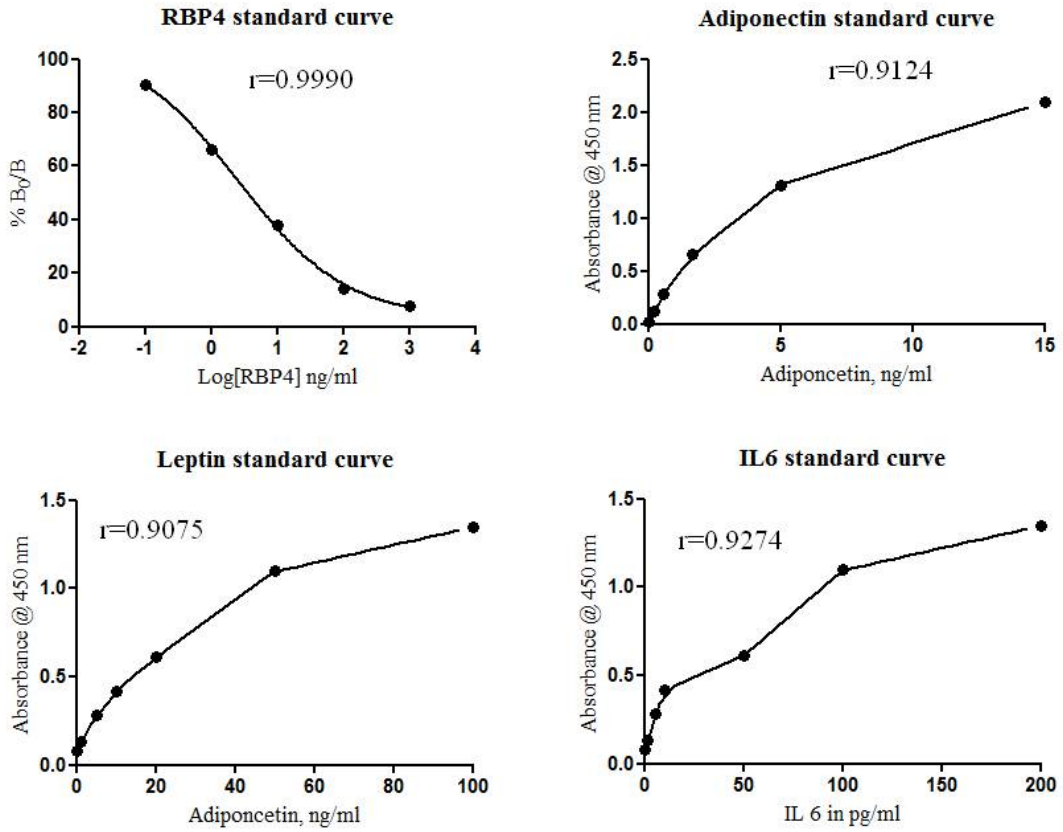


Figure 5-3: Typical standard curve obtained for various adipokines studied

5.5 Statistical analysis

5.5.1 Sample power calculation

- *Adiponectin:* Studies from IDPP-1[301], and Funagat[317] found a strong inverse association between adiponectin and incident T2DM. From our IDPP-1 cohort study, the mean baseline adiponectin was lower in the T2DM group as compared with normoglycaemic group (11.7 ± 5.5 vs. 16.7 ± 7.6 µg/ml). With 50 individuals in each group, this expected difference, and with these expected standard deviations, can be demonstrated as significant at $p<0.05$ and with 88% power.
- *Retinol binding protein 4 (RBP4)*: From a previous cross sectional study in China [318] it was reported that the mean levels of RBP4 were higher in T2DM individuals than in the normoglycaemic group (T2DM: 30±11 µg/ml; normal: 24±7 µg/ml). With 53 participants in each group, and with these standard deviations, the expected difference can be demonstrated as significant at 5% significance and 80% power.
- *Interleukin 6 (IL-6)*: The European Prospective Investigation into Cancer and Nutrition (EPIC)–Potsdam study demonstrated that IL-6 was elevated in T2DM compared to controls and this study also evidenced that combined elevation of interleukin 1 β and IL-6 predict incident T2DM rather than the single variable [319]. With 90 individuals in each group, the expected difference with these standard deviations [1.67 ± 1.59 vs. 2.45 ± 1.80 pg/ml [319]], can be demonstrated as significant at $p<0.05$ and 80% power for IL-6.
- Leptin: The British Regional Heart Study[305], demonstrated that leptin was elevated in T2DM individuals than in the non-diabetic group (T2DM: 14.3 (IQR: 10.2-21.9); normal: 8.93 (IQR: 10.2-21.9); assumed standard deviation: 11.5; $P<0.0001$). With

72 individuals each group, and with these standard deviations, the expected difference can be demonstrated as significant at 5% significance and 80% power.

In total for this study, 76 cases and 71 controls were studied for biomarker analysis.

5.5.2 Statistical methods

Normally distributed variables were expressed as mean ± SD and compared using two-tailed student t-test. Non-parametric variables were expressed as median (25^{th} and 75^{th} quartile range) and compared using Mann Whitney test. Before statistical analysis, non-normally distributed parameters were natural logarithmically transformed to approximate a normal distribution. Pearson correlation and multiple linear regression analysis (stepwise addition) were performed to evaluate the association of biomarkers with surrogate insulin measures and cardiovascular risk factors. Multiple logistic regression models were used to assess the multivariable-adjusted relative risk for the development of diabetes. The power of each biomarker to predict a progression to diabetes was assessed with a receiver-operating-characteristic (ROC) curve. The area under the curve (AUC) of the ROC and a 95% confidence interval (CI) were calculated by the Delong [216] method to evaluate the diagnostic utilities of the marker for diabetes prediction. The optimal cut-off point of each marker was estimated by calculating the Youden index [217]. Then, its specificity, sensitivity were calculated using that cut-off. The positive likelihood ratio was calculated using the formula: sensitivity / (1-specificity). For this assessment, we have created three models: 1) a univariate biomarker model to evaluate the predictive power of each biomarker in separate analysis; 2) multivariable model (FPG + biomarker) using a logistic regression equation with those biomarkers as explanatory variables for predicting diabetes; 3) the combination of non-glycaemic

biomarkers which was shown to be associated with diabetes in step-1. For each curve, a test for the equality of the AUC of ROC between FPG and the additional model was evaluated using an algorithm suggested by DeLong et al [216]. Statistical analysis was performed using SPSS version 19.0 (IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp).

5.6 Results:

5.6.1 Characteristics of the participants:

Participant characteristics are presented in **Table-5.1**. Mean age and BMI of the cohort was 47.1 ± 4.7 years and 26.0 ± 3.0 Kg/m^2 respectively. As compared to non-progressors (NGT+IGT group), the mean values of diastolic blood pressure (P=0.046), fasting plasma glucose (FPG), 30 minutes glucose, 2-hr post glucose (2-hr PG) and HbA1c (P<0.0001 for all), and triglycerides (P = 0.022) were significantly higher in progressors. Accordingly, progressors were more insulin resistant (P<0.0001) and had decreased β-cell function (P<0.0001) as compared to non-progressors.

5.6.2 Biomarker levels

The baseline levels of adiponectin was decreased by 30% in those who progressed to T2DM (non-progressors: 16.4 (IQR: 11.6-21.4) vs. progressors: 11.5 (IQR: 8.6-15.0); P<0.0001). The baseline GGT activity was increased by 55% ((22.0 (IQR: 17.2-35.8) vs. 34.1 (IQR: 24.7-56.0); P<0.0001)) in participants who progressed to T2DM. The levels of RBP4 ((17.3 (IQR: 13.1-21.0) vs. 21.3 (IQR: 17.7-24.9); % change: 23.1%; P=0.010)) and IL-6 ((5.3 (IQR: 4.7-12.4) vs. 14.9 (IQR: 5.8-24.9); %change: 181.0%; P<0.0001)) were increased in individuals who progressed to diabetes compared to non-progressors. The levels of leptin and vitamin D3 did not differ between the groups.

Table 5-1: Baseline characteristics of individuals stratified based on glycaemic categories

Variables	Non-progressors n=76	Progressors n=71	P value
Age (years)	47.7±4.8	46.7±4.6	0.217
Body mass index, Kg/m^2	25.9±2.7	26.2±3.5	0.491
Waist circumference, Cm	92.6±7.1	93.5±7.4	0.465
Total body fat, %	26.8±5.5	27.2±5.2	0.683
Systolic blood pressure, mmHg	122.8±16.2	124.5±14.6	0.519
Diastolic blood pressure, mmHg	79.2±10.1	82.4±8.6	0.046
Fasting plasma glucose, mmol/l	5.39±0.50	5.82±0.58	<0.0001
2-hr plasma glucose, mmol/l	8.42±0.65	9.28±0.88	<0.0001
HbA1c, %	6.0±0.3	6.3±0.4	<0.0001
Triglycerides, mmol/l [a]	1.49 (1.09-2.05)	1.84 (1.32-2.33)	0.022
Cholesterol, mmol/l	5.01±0.90	4.93±0.92	0.604
HDL cholesterol, mmol/l	0.91±0.26	0.87±0.23	0.335
GGT, UL^{-1} [a]	22.0 (17.2-35.8)	34.1 (24.7-56.0)	<0.0001
HOMA-IR	3.0±1.3	3.7±1.3	<0.0001
Insulinogenic index [a]	62.7 (43.5-97.0)	34.5 (23.7-52.0)	<0.0001
Disposition index	187.0(144.9-204.3)	113.9(95.1-138.3)	<0.0001
Adiponectin, µg/ml [a]	16.4 (11.6-21.4)	11.5 (8.6-15.0)	<0.0001
IL-6, pmol/ml [a]	5.3 (4.7-12.4)	14.9 (5.8-24.9)	<0.0001
Leptin, ng/ml [a]	8.9 (6.8-16.5)	12.3 (8.0-17.4)	0.170
RBP4, µg/ml [a]	17.3 (13.1-21.0)	21.3 (17.7-24.9)	0.010
Vitain D3, nmol/ml	40.9 (24.7-56.6)	44.0 (23.7-58.3)	0.797
Baseline energy intake (Kcal)	2069 (1918-2292)	2091 (1880-2277)	0.729
Baseline physical activity	36.0 (21.8-46.0)	39.0 (23.0-50.0)	0.617

Legend: The data are expressed as mean ± SD for normally distributed variables – P value computed by two-tailed t-test

[a]: data expressed as median (inter quartile range) – P value computed by Mann-Whitney test.

5.6.3 Correlates of biomarkers

Correlations of biomarkers with core metabolic and anthropometric risk factors are shown in **Table 5-2**. IL-6, leptin and RBP-4 were positively correlated with BMI whereas adiponectin was negatively correlated. Leptin, RBP-4 and GGT were significantly positively whereas adiponectin was inversely correlated with HOMA-IR. RBP-4 and leptin were positively correlated with HbA1c levels. GGT and RBP-4 were positively and strongly correlated with triglycerides levels (P<0.0001). In contrast, vitamin D3 levels did not correlate with the classical and core risk factors of dysglycaemia. There were extensive inter-correlation between biomarkers.

Table 5-2: Pearson coefficient correlation between adipokines and risk factors for diabetes

Variables	Adiponectin	IL-6	Leptin	RBP-4
Adiponectin (µg/ml)*	--	-0.057	-0.121	-0.124
IL-6 (pmol/ml)*	-0.057	--	0.277‡	0.160
Leptin (ng/ml)*	-0.121	0.277‡	--	0.204†
RBP-4 (µg/ml) *	-0.112	0.122	0.204†	--
Age (years)	0.166†	-0.065	0.024	-0.043
Body mass index (Kg/m^2)	-0.238†	0.169†	0.433‡	0.303‡
Waist circumference (Cm)	-0.126	0.174†	0.349‡	0.272†
Fat%	-0.052	0.221†	0.275†	0.145
Systolic blood pressure (mmHg)	-0.114	-0.006	0.146	0.183†
Diastolic blood pressure (mmHg)	-0.084	0.139	0.228†	0.181†
Fasting glucose (mmol/l)	-0.192†	0.119	0.021	0.245†
2-hr glucose (mmol/l)	-0.154	0.194†	0.280†	0.102
HbA1c (%)	-0.115	0.027	0.153	0.256‡
Triglycerides (mmol/l)*	-0.008	0.045	0.154	0.314‡
HDL cholesterol (mmol/l)	0.234†	0.068	0.063	-0.077
HOMA-IR*	-0.211†	0.080	0.222†	0.242†
Insuliongenic index (pmol/mmol)*	0.088	-0.153	0.185†	0.036

*: Log transformed; †: P<0.01; ‡: P<0.001; IL-6: Interleukin 6; RBP4: retinol binding protein 4;

5.6.4 Association of biomarkers with incident diabetes:

The association of adipokine markers with incident diabetes is shown in **Table-5.3**. Adiponectin (OR: 0.86 [95%CI: 0.80-0.93]; P<0.0001), IL-6 (OR: 1.04 [1.01-1.07]; P=0.015), and RBP4 (OR: 1.06 [95%CI: 1.00-1.13]; P=0.048) were significantly associated with T2DM even after adjustment for potential confounders including age, BMI, body fat% and HOMA-IR . Leptin and vitamin D3 was not associated with diabetes in our cohort in an unadjusted model. The inclusion of all six novel risk factors: adiponectin, leptin, RBP-4 and IL-6, along with GGT and HTWP, which were identified as significant predictors in **chapter-4**, in a logistic regression model showed that raised levels of IL-6 (OR: 1.05 [95%CI: 1.01-1.09]; P=0.010), GGT (OR: 1.04 [95%CI: 1.01-1.05]; P=0.010) and lower levels of adiponectin (OR: 0.86 [95%CI: 0.79-0.95]; P=0.006) were independently associated with incident diabetes in our cohort (**Table 5-4**).

Table 5-3: Adjusted relative risk (RR) of incident type 2 diabetes by of adiponectin, IL-6, leptin, GGT and RBP4

	Unadjusted			**Adjusted***		
	β (s.e)	OR [95%CI]	P value	β (s.e)	OR [95%CI]	P value
Adiponectin (µg/ml)	-0.137 (0.034)	0.87 [0.82-0.93]	<0.0001	-0.150 (0.040)	0.86 [0.80-0.93]	<0.0001
IL-6 (ng/ml)	0.037 (0.014)	1.04 [1.01-1.07]	0.010	0.037 (0.015)	1.04 [1.01-1.07]	0.015
Leptin (ng/ml)	0.022 (0.022)	1.02 [0.98-1.07]	0.314	---	---	---
GGT (IUL^{-1})	0.021 (0.008)	1.02 [1.01-1.04]	0.008	0.019 (0.010)	1.02 [1.00-1.04]	0.042
RBP4 (µg/ml)	0.073 (0.025)	1.08 [1.02-1.13]	0.004	0.059 (0.030)	1.06 [1.00-1.13]	0.048
VitaminD3 (nmol/ml)	-0.005 (0.007)	1.00 [0.98-1.01]	0.437	---	---	---

Dependent variable: diabetes vs. non-diabetes; odds ratios computed from logistic regression analysis; β: regression coefficient; OR: odds ratio

***** The covariates which showed significant univariate associations with biomarkers were chosen for the multivariate analyses.
-Adiponection: age, body mass index, HDL-C and HOMA-IR.
-IL-6: Fat%, leptin
-RBP4: BMI, systolic blood pressure, triglycerides, GGT, leptin and HOMA-IR
Leptin and vitamin D3 did not show an association with incident diabetes in an unadjusted analysis. Hence, an adjusted model was not explored.
If the biomarkers showed a multiple correlation with anthropometric measures (BMI, waist circumference, Fat%), or haemodynamic measures (SBP, DBP) whichever showed a strongest association alone was chosen for the modeling to minimize co-linearity.
Glycaemic markers were not included as covariates because participants were selected according to their having raised glycaemia.

Table 5-4: Adjusted relative risk of incident type 2 diabetes when all the biomarkers were included as a single model

Variable	Unadjusted		Adjusted*	
	OR [95%CI]	P value	OR [95%CI]	P value
Adiponectin	0.86 [0.80-0.93]	<0.0001	0.86[0.79-0.95]	0.006
IL-6	1.04 [1.01-1.07]	0.006	1.05[1.01-1.09]	0.010
GGT	1.02 [1.01-1.04]	0.013	1.04[1.01-1.06]	0.006

Legend:

Dependent variable: diabetes vs. non-diabetes.

Non-significant variables: RBP4, leptin and HTWP; Leptin was included in the model because of its multiple correlation with biomarkers.
Equation: Ln[odds ratio]= -0.151 (adiponectin) + 0.040 (IL-6) + 0.022 (GGT)

Candidate predictors identified (in **Table-5.3**) were entered together for the final prediction model using multiple logistic regression analysis (probability to enter=0.01; probability to remove=0.2 to protect against type 1 errors); *adjusted for age, family history of diabetes, HOMA-IR, insulinogenic index, HbA1c; the predictive probability (β coefficient) of that model was saved for the ROC analysis.

5.6.5 Predictive power of biomarkers for progression of diabetes

The predictive ability of each marker individually and its corresponding optimal cut-off point is shown in **table 5-5**. The AUC's to predict the progression to diabetes among glycaemic biomarkers were 0.708 (95%CI: 0.625-0.791) for FPG, 0.779 (95%CI: 0.704-0.844) for 2-hr PG and 0.725 (95%CI: 0.643-0.808) for HbA1c. As expected, glycaemic markers were the best predictors for diabetes prediction. Amongst the biomarkers, IL-6 displayed highest predictive value (AUC: 0.719 (95%CI: 0.635-0.803)), followed by adiponectin (AUC: 0.695 (95%CI: 0.613-0.768)) and RBP4 (AUC: 0.658 (95%CI: 0.571-0.749)).

Table 5-6 shows the improvements in predictive power of the models when the adipokines when combined with FPG (base model) as derived from the logistic regression analysis. Significant improvements were achieved by including adiponectin or IL-6 in the model. The results showed that the model comprised of FPG + adiponectin was effective in identifying individuals at high risk of diabetes (AUC: 0.774 (95%CI: 0.698-0.839); P=0.034; sensitivity (%): 76.1 (95%CI: 64.5-85.4); specificity (%): 71.1 (95%CI: 59.5-80.9); positive likelihood ratio: 2.63). Though, IL-6 was equally effective in identifying incident diabetes the sensitivity was lower (sensitivity (%) 65.8 (95%CI: 54.0-76.3)) compared with adiponectin. The addition of RBP-4 above FPG did not statistically improve the predictive power in our analysis. The combination of non-glycaemic biomarkers (AUC: 0.761 (95%CI: 0.684-0.838)) alone did not show substantial improvement to predict diabetes compared with FPG + biomarker model.

Table 5-5: Area under the receiver operating characteristics and predictabilities of single markers for progression of diabetes

Biomarker	AUC (95% CI)	P value	Optimal cut-off point [a]	Sensitivity % (95% CI)	Specificity % (95% CI)	Positive likelihood ratio
Non-glycaemic						
Adiponectin	0.695(0.613-0.768)	<0.0001	≤ 14	67.6 (55.5-78.2)	63.2 (51.3-73.9)	1.84
IL-6	0.719(0.635-0.803)	<0.0001	≥ 7.2	70.4 (58.4-80.7)	69.7 (58.1-79.8)	2.33
GGT	0.694(0.608-0.781)	<0.0001	≥23.3	80.3(69.1-88.8)	59.2(47.3-70.4)	1.97
RBP4	0.658(0.571-0.749)	0.001	≥ 14.2	69.0 (56.9-79.5)	55.3 (43.4-66.7)	1.57
Glycaemic						
FPG	0.708(0.625-0.791)	<0.0001	≥ 98.0	71.8 (59.9-81.9)	60.5 (48.6-71.6)	1.82
OGTT 2-hr glucose	0.779(0.704-0.844)	<0.0001	≥ 157.0	67.6 (55.5-78.2)	77.6 (66.6-86.4)	3.02
HbA1c	0.725(0.643-0.808)	<0.0001	≥ 6.0	77.5 (66.0-86.5)	57.9 (46.0-69.1)	1.84

AUC: area-under-curve; CI: confidence interval; IL-6: interleukin-6; RBP4: retinol binding protein 4; FPG: fasting plasma glucose; The units of each optimal cut-off point was shown in Table-6.1

Table 5-6: The area under the receiver operating characteristics and predictabilities of multiple markers for progression of diabetes by logistic regression models

Multiple biomarkers	AUC (95% CI)	Incremental AUC above 0.5	P value[b]	Sensitivity % (95% CI)	Specificity % (95% CI)	Positive likelihood ratio
FPG [a]	0.708 (0.625-0.791)	--	--	71.8 (59.9-81.9)	60.5 (48.6-71.6)	1.82
FPG+IL-6	0.775 (0.698-0.839)	0.07	0.0323	77.5 (66.0-86.5)	65.8 (54.0-76.3)	2.26
FPG+RBP4	0.723 (0.641-0.792)	0.01	0.5347	52.1 (39.9-64.1)	80.3 (69.5-88.5)	2.64
FPG+Adiponectin	0.774 (0.698-0.839)	0.07	0.0374	76.1 (64.5-85.4)	71.1 (59.5-80.9)	2.63
FPG+GGT	0.740 (0.660-0.819)	0.03	0.1957	71.8(59.9-81.9)	67.1(55.4-77.5)	2.18
FPG+HbA1c	0.768 (0.692-0.845)	0.06	0.0551	81.7(70.7-89.9)	64.5(52.7-75.1)	2.00
Adiponectin+ GGT+IL-6	0.761 (0.684-0.838)	0.05	0.3432	85.9(75.6-93.0)	63.2(51.3-73.9)	2.33

Legend: AUC: area-under-curve; CI: confidence interval; IL-6: interleukin-6; RBP4: retinol binding protein 4; FPG: fasting plasma glucose; a: FPG is the base model; b: P value was for comparing the AUC between base model (FPG) and additional models with multiple markers (FPG+ adipokine marker).

5.7 Conclusions

In this case-control cohort study in middle-aged Asian Indian men with prediabetes, participants with low levels of adiponectin, and high levels of IL-6, GGT and RBP4 had a higher risk for development of diabetes independent of age, obesity and a comprehensive array of other potential confounders or explanatory factors. The effect persisted even after the adjustment for HOMA-IR and insulinogenic index.

As reported in the recent meta-analysis by Li et.al [304], higher adiponectin levels have been shown to be consistently shown to be associated with incident diabetes after multivariate adjustment across a range of populations. Consistent with our previous findings [301] this study also provides evidence that adiponectin could be a predictive marker for identification of T2DM in Asian Indian individuals; the predictive power persists even after the multivariate adjustments. These observations corroborate the validity and usefulness of adiponectin as an independent baseline or cross-sectional measure of metabolic status in general and of diabetes risk in particular. This study also shows that HOMA-IR was inversely associated with adiponectin. A similar relationship was also reported previously in Asian Indian adult men and women [320] but not in non-diabetic teenagers [321]. Adiponectin, an anti-inflammatory cytokine produced by adipose tissue, is an important regulator of insulin sensitivity in skeletal muscle and liver [322-324]. In addition to its beneficial effects on insulin sensitivity, adiponectin also has direct antiatherogenic and anti-inflammatory effects [323].

Amongst the biomarkers studied in this cohort, baseline levels of IL-6 showed the strongest associations with incident diabetes. These findings support the hypothesis that there exists an association of chronic inflammation and development of diabetes. Inflammation both predicts and precedes diabetes[325]. Although raised IL-6 levels have been previously associated with subsequent diabetes in multi-ethnic

populations[305,307,326], the exact mechanism of its action remains to be unravelled. It is important to study the link between chronic inflammatory mediators and insulin resistance because, low-grade systemic inflammation could mediate higher diabetes risk via insulin resistance [327], but several observations also suggest that the independent association of IL-6 with diabetes operates through alternative mechanisms [305,326,328]. In accordance with our findings, in the BRHS study, the relationship of IL-6 was independent of measures of insulin resistance in aged men [305]. At present, there is no evidence for an independent role of IL-6 in impaired β-cell function/apoptosis. A comprehensive review by Kristiansen OP and Mandrup-Poulsen[329] concluded that chronically elevated IL-6 levels might contribute to the development of T2DM via mechanisms including altered insulin signalling in hepatocytes/adipocytes and effects on the central nervous system to impair energy regulation. Further observations are required to identify the causality of IL-6 and diabetes.

In this case-control cohort study, baseline serum RBP-4 levels were independently associated with incident diabetes after adjustments for known metabolic risk factors including GGT, triglycerides, BMI and HOMA-IR. Previously, a large cross-sectional, general population study showed a strong association of RBP4 with prediabetes[315]. Recently, a prospective association of RBP4 with incident diabetes over 9 years was observed in a population-based cohort in women (HR = 1.68 [95%CI 1.00 – 2.82] but not in men [316]. In addition, cross-sectional studies [312,330] conducted in middle-aged Chinese adults also showed a strong positive association between RBP4 and impaired glucose regulation. The present analysis provides new evidence on the link between RBP4 and incident diabetes in Asian Indian individuals with IGT.

The serum RBP4 levels were positively associated with cardiometabolic risk factors, especially increased levels of triglycerides in this cohort. These findings were in line

with previous studies [297,313,330]. Regarding the mechanism underlying the association between RBP4 and triglycerides, a direct effect of RBP4 on hepatic fat accumulation may be plausible[294]. In addition, production of triglycerides in the liver and release of very low-density lipoprotein into the circulation generally increase in an insulin-resistant state, and this may also partially explain the close nexus between RBP4, triglycerides and insulin resistance. On the contrary other studies failed to show an association between RBP-4 and insulin resistance[295,331]. Though the serum RBP-4 level correlated with BMI, the relationship between the serum RBP-4 and insulin resistance was independent of obesity. It is conceivable that contrary to those of other populations[298,313] liver could be the primary source of RBP-4 in circulation in Asian Indians.

In this study, the levels of serum RBP-4 were relatively low compared with those reported in Caucasians [313,315,316,332] and were closely matched with those observed in Chinese [330], Japanese [297] and Korean [312] adults. A plasma concentration of approximately 24-79 μg/ml RBP-4 was reported in normal lean Japanese individuals[333]. But, in this cohort, the levels of RBP-4 were lower even in those who developed T2DM. It may be possible that the assay used in this study underestimated the RBP-4 level, compared with methods such as quantitative Western blotting and this could account for the varied results reported by different laboratories[334]. Further study is needed to investigate ethnic variations among RBP-4 measurement methods.

To summarise, the aforementioned findings suggest at least three divergent areas in the pathophysiology of diabetes. Firstly, the association of adiponectin with incident diabetes has been well-documented: adiponectin increases insulin sensitivity, improves glucose tolerance and inhibits inflammation. Secondly, the association of GGT with diabetes could be due to the excessive lipid accumulation in hepatocytes and the

resultant hepatic insulin resistance possibly related to decreased portal insulin extraction and increased glucose output, thereby contributing to the development of total body insulin resistance and diabetes. Thirdly, IL-6 was associated with diabetes independent of insulin resistance and other diabetogenic risk factors. These findings are obviously important in considering the pathogenesis of diabetes.

In this study additive value of non-glycaemic markers over and above FPG was also evaluated. Though, the combination of non-glycaemic biomarkers with FPG improved the diagnostic power, the predictive utility was seen to be very marginal. Neither did the combination adiponectin, IL-6 and GGT substantially improve the diagnostic predictability above glycaemic measures. This clearly demonstrates that the utility of non-glycaemic biomarkers may offer little or marginal improvement to predictive precision when added to the glycaemic markers. In line with our study, Salomma et al [335] showed an marginal, non-significant improvement when adding the novel biomarkers, adiponectin, apolipoprotein B, interleukin 1-receptor A and ferritin, to the classical risk factors, age, BMI, sex, HDL-C, non-HDL-C, TG, systolic blood pressure, antihypertensive medication, current smoking, blood glucose, and history of CVD at baseline, in predicting T2DM in high risk middle aged populations from the FINRISK97 cohort over 5 years.

Amongst the markers studied in this cohort, baseline disposition index, a measure of β-cell function was the most discriminatory measure associated with incident diabetes (explained in detail in **chapter-4**). In the present analysis, the combination of FPG+HbA1c did not incrementally improve the AUC ROC in predicting diabetes compared with individual glycaemic measures. It should be noted that the individuals were prediabetic at baseline; accordingly, the narrow range of FPG and HbA1c our study encompassed, with little variation among individuals, may have limited our

ability to detect a significant relationship. However, other prospective studies clearly demonstrated a predictive utility of adding HbA1c with FPG [336].

As regards leptin's link with diabetes, we have demonstrated that the levels of this adipokine do not predict incident diabetes in a completely adjusted model. A similar result was also observed in the Health, Aging, Body Composition (Health ABC) study, which demonstrated that after controlling for BMI, visceral fat, fasting glucose, fasting insulin, high-density lipoprotein cholesterol, triglycerides, and hypertension at baseline, leptin was not associated with incident diabetes [337]. In fact, much of the association between leptin and subsequent diabetes could be accounted for by obesity and insulin resistance[338].

The main strength of the analysis includes simultaneous measurements of multiple biomarkers and the study cohort comprised of a sample derived from Asian Indian individuals. However, the study has a few limitations. Firstly, the association between biomarkers with diabetes observed in this study was derived from a small sample size. However, the study design was adequately powered to detect the association. Secondly, we included only men in our study. The pathogenesis of biomarkers with diabetes needs to be ascertained in women. Thirdly, though we included a comprehensive array of biomarkers we do miss out several potential candidates such as C-reactive protein, markers of endothelial dysfunction and oxidative stress which may affect the predictive ability of these proposed biomarkers. Finally, we did not assess the prevalence of non-alcoholic fatty liver disease in this cohort to ascertain the causal link between fatty liver and diabetes.

To summarise, we have demonstrated that combination of adiponectin, IL-6 and GGT independently associated with diabetes. But, these markers have little additive value over and beyond glycaemic markers for the prediction of diabetes. It can be noted that

non-glycaemic biomarkers have a role in understanding the underlying pathogenesis of diabetes, but the “classical” glycaemic biomarkers seem to be sufficient as a risk marker in clinical practice.

6 SUMMARY

The overall summary of the thesis is presented below:

6.1 Chapter-3 – Trial findings

6.1.1 What is already known on this topic?

- According to the recent International Diabetes Federation (IDF) estimates, India accounts for around 31.9 million with undiagnosed diabetes. Unlike western populations, Asian Indians develop diabetes at a younger age, mostly in the working age group. Hence, identification and prevention of diabetes at this age group is of great public health importance.
- Findings from several multi-ethnic, randomized controlled trials across all geographic spectrums including Indian Diabetes Prevention Progarmme-1 have consistently established that T2DM can be postponed or delayed by lifestyle modification in prediabetic individuals by repeated motivational strategies.
- Mobile phone messaging can be a powerful medium for health communication. Though, its role in adherence to antiretroviral therapy, smoking cessation, asthma and for malarial treatment adherence is already established, its effectiveness in the prevention of diabetes has not been studied so far in a prospective setting.

6.1.2 The novelty of the data presented and its impact on the field

- Mobile phone-based text messaging can be an effective technique for disseminating healthy lifestyle modification principles. The cumulative incidence of T2DM was significantly reduced in the intervention group compared with the standard-care group (HR: 0·64, 95% CI 0·45–0·92; p= 0·015).
- The benefit of intervention was independent of body weight reduction. The intervention group showed an improvement in dietary compliance levels compared with the standard care group.
- Our results demonstrated initial evidence that motivation through SMS may be a highly effective and acceptable tool to substantially reduce incidence of diabetes. According to our finding, 11 (95% CI 6–55) individuals with IGT must be provided with such an intervention for two years to prevent one case of diabetes.

6.2 Chapter-4A: Liver enzymes and incident diabetes

6.2.1 What is already known on this topic?

- Evidence from several prospective studies in multi-ethnic populations provides a strong link between elevated liver enzymes such as gamma-glutamyl transferase (GGT) and alanine transaminase (ALT) and development of diabetes.
- The objective of this analysis was to find out the association of liver enzymes with the prediction of incident diabetes in Asian Indians.

6.2.2 New findings of this analysis

- Baseline GGT, but not ALT was significantly higher in incident diabetes cases. The levels of GGT decreased in individuals who reverted to normal glucose tolerance (NGT), whereas it increased in individuals who deteriorated to diabetes ($P<0.0001$).
- The risk of T2DM significantly increased with increasing baseline GGT after adjusting for confounders (HR: 2.02 [95%CI: 1.35-3.02]; P=0.001).
- Receiver operating characteristic curve showed that the model comprising of baseline fasting plasma glucose and GGT was as sensitive in identifying individuals with risk of diabetes as 2-hr plasma glucose (2-hr PG) and HbA1c.
- GGT is an independent predictor of incident diabetes. The combination of GGT and HbA1c offers a simple and sensitive tool to identify individuals at high risk of diabetes.

6.3 Chapter -4B: Hypertriglyceridemic Waist Phenotype and the Risk of Incident Diabetes in Asian Indian individuals with IGT

6.3.1 What is already known on this topic?

- Hypertriglyceridemic waist phenotype (HTWP) is known to be associated with insulin resistance and can be used as a simple proxy for insulin resistance.
- The prevalence of central adiposity and hypertriglyceridemia are particularly high in Asian Indian descendants.
- Though, cross-sectional studies have demonstrated an association of the HTWP phenotype with diabetes, the utility of this measure for the prediction of incident diabetes has not been much studied.
- This cohort analysis was done with the objective of analyzing the possible association of HTWP with incident diabetes, considering the simplicity of identifying the presence of HTWP and its association with other cardio-metabolic risk factors.

6.3.2 The novelty of the data presented and its impact on the field

- HTWP was strongly associated with insulin resistance: (β: 0.23 (0.09-0.37); P=0.01).
- In this study among Asian Indian men with impaired glucose tolerance (IGT), presence of HTWP at baseline was found to predict incident diabetes, over the two year follow-up period (HR: 1.49 [95%CI: 1.01-2.21]; P=0.047) after adjusting for age, study group, BMI, total body fat percentage, family history of diabetes, hypertension, smoking and drinking, 2-hr PG, total and HDL-C and GGT levels. However, the predictive power was abolished after the addition of HOMA-IR into the model (HR: 1.39 [0.92-2.10]; P=0.119).
- HTWP offers a simple, alternate tool to help to screen individuals with increased risk of incident diabetes and the association is mediated by insulin resistance.

6.4 Chapter-4C: Disposition index and diabetes

6.4.1 What is already known on this topic?

- There exists an inverse relationship between insulin sensitivity and insulin secretion and the product of these measures approximates a constant, termed the "*Disposition Index*".
- Several prospective and cross-sectional studies have shown that disposition index is an early marker of inadequate β-cell compensation and predicts incident diabetes. So far no prospective study had shown this association in Asian Indian individuals.
- Therefore the objective of this analysis was to elucidate the association of a disposition index derived from the oral glucose tolerance test (OGTT) measures with incident diabetes.

6.4.2 The novelty of the data presented and its impact on the field

- The product of the OGTT area-under-curve (AUC) insulin to AUC glucose from 0 to 120 min and insulin sensitivity index (ISI) was the best equation to depict disposition in this cohort (Y=-0.9642*X+4.982).
- Individuals with IGT with good β-cell compensation at baseline were least likely to develop diabetes.
- In this cohort, decreased dietary intake and minimal weight reduction significantly improved the β-cell function.
- The present analysis demonstrated that the OGTT-derived measures of disposition index may be an alternate surrogate composite measure to predict incident diabetes. Furthermore, improving insulin sensitivity by caloric restriction and minimal weight reduction may have beneficial effects on β-cell function and prevent diabetes for relatively long periods of time in individuals with genetic susceptibility for the disease.

6.5 Remarks – Classical predictors:

Among the classical markers studied, disposition index (AUC: 0.717 (95%CI: 0.675-0.756); sensitivity (%): 70.0 (95%CI: 61.0-78.0); specificity (%): 64.1 (95%CI: 59.0-68.9)) was a better predictor of incident diabetes compared with other OGTT based insulin measures such as HOMA-IR (AUC: 0.642 (95%CI: 0.598-0.685); sensitivity (%): 59.2 (95%CI: 49.8-68.0); specificity: 62.7 (95%CI: 57.6-67.6)) and insulinogenic index (AUC: 0.599 (95%CI: 0.554-0.642); sensitivity (%): 45.0 (35.9-54.3); specificity (%): 72.5 (67.7-77.0)). However, this measure requires performance of the 3 sampling OGTT with estimations of plasma insulin and glucose which is labour intensive test. We propose that combination of HbA1c and GGT may be an alternate, simple measure to identify high-risk individuals in this cohort.

6.6 Chapter-6: Novel biomarkers and diabetes

6.6.1 What is already known on this topic?

- There is scarce information available on the association of non-glycaemic biomarkers with diabetes in Asian Indian individuals.
- This chapter examined the evidence for associations between novel biochemical markers including adiponectin, leptin, inerleukin-6 (IL-6), retinol binding protein-4 (RBP4), vitamin D3 with T2DM.
- Also, the additive utility of non-glycaemic biomarkers over and above that of glycaemic measures in identifying individuals with diabetes was also assessed.

6.6.2 The novelty of the data presented and its impact on the field

- The present analysis demonstrates that the serum level of lower adiponectin (OR: 0.860 [95%CI: 0.800-0.925]; P<0.0001), higher IL-6 (OR: 1.041 [95%CI: 1.012-1.071]; P=0.006) and GGT (OR: 1.022 [95%CI: 1.005-1.040]; P=0.013) were associated with diabetes.
- Serum RBP4 levels showed a modest association with diabetes (OR: 1.061 [95%CI: 1.001-1.125]; P=0.048). But, in a multiple biomarker model its association was attenuated.
- Serum leptin and Vitamin D3 were not associated with diabetes.
- Though, these biomarkers provided may offer novel information regarding mechanisms of diabetes pathogenesis, they did not improve the predictive power over and above that of glycaemic measures in identifying individuals with diabetes.
- Nevertheless, the non-glycaemic biomarkers may have a pathogenic role in the development of diabetes.

6.7 Overall conclusion

The existing primary prevention programmes for T2DM are resource-intensive, labour expensive and it is hard to translate the results from a research setting to the community at large. The present trial affirms that delivery of lifestyle intervention content through mobile phone based text messaging service may be an alternate means to disseminate healthy lifestyle principles in the field of preventive medicine. Also, it is important to identify simple, cost-effective screening tools to identify high-risk individuals requiring intervention. Most importantly, identification of novel biochemical markers from various organs such as the liver and adipose tissue provides important information regarding their pathological links with diabetes. This dissertation serves to evaluate the associations of routine clinical variables and novel biomarkers with T2DM among high-risk individuals, and supports that routine clinical measures are sufficient to predict diabetes in this cohort. However, the non-glycaemic biomarkers have a role in understanding the pathogenesis of diabetes.

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8 APPENDICES

Appendix - 1

PARTICIPANT INFORMATION PAMPHLET

Proposal Title: Role of information technology in the prevention of diabetes

Proposal Version: 1.0

Principal Investigator/ Address: Dr. A. Ramachandran, India Diabetes Research Foundation and Dr. A. Ramachandran's Diabetes Hospitals, No. 28 Marshalls Road, Egmore, Chennai 600 008.

Project Identification Number: UKIERI-IDRF/08/039

Funder: UK-India Education and Research Initiative (IND/CONT/06-07/187E)

Participant ID:

1. Why is this programme conducted?

India has a large population with diabetes in the working age group and the number is increasing disproportionately. The rates of urbanization, migration from rural to urban areas and adoption of modern lifestyle have resulted in reduced physical activity and unhealthy diet habits. Diabetes is preceded by subclinical forms of increased blood glucose levels, known as prediabetes or Impaired Glucose Tolerance (IGT). People with prediabetes have a high risk of developing diabetes. India has a large number of persons with IGT.

Primary prevention of type 2 diabetes by lifestyle modification is a possible solution to postpone the rising epidemic of the disease. Our team has already conducted 2 large primary prevention programmes which have shown that diabetes is largely preventable. Now, in this programme, we are trying to prevent diabetes in a large group of persons at risk of developing the disease.

This community-based programme will be conducted in various industrial townships and commercial organizations located in Tamil Nadu and other parts of South India. We plan to include 514 participants, both men and women. The project proposal and this consent form have been carefully reviewed and approved by the independent Ethics Committee, India. These committees are set-up to protect the rights and well-being of the study participants and they will closely monitor the study progress at regular intervals.

2. What will happen when you participate in this programme?

You are being asked to participate in the Indian Diabetes Prevention Programme because you are aged between 35-55 years with a high risk of developing diabetes in the future. It is important that you read the information about the programme and understand what you will be asked to do if you decide to be in the study. You also need to understand the possible benefits and inconveniences of participation. It you decide to participate, you will need to sign this form, which states that you have given consent to participate. Feel free to discuss this with your employer or family member / friend before you take your decision.

3. Details of the Programme:

During the initial screening your 2 hr post glucose value was in a prediabetic range (2hr post glucose value: ≥ 160- ≤ 199 mg/dl).

Enrollment Visit: In this confirmatory test you will undergo the standard oral glucose tolerance test (oral glucose tolerance test (OGTT, glucose load 75g) at fasting and at 30 and 120 minutes after 75g glucose ingestion). Based on the result the following inference will be made. If the value is between 140-199 mg/dl, you will be enrolled in this programme.

Range	Interpretation
<140 mg/dl	Normal
140-199 mg/dl	Eligible to participate in the study (High Risk)
≥ 200 mg/dl	Referred to physician

You will be required to come in fasting state for this visit. During this visit you will be assigned to either the intervention group or to usual care. Participants assigned to usual care will have an initial interview conducted by the IDRF team, delivering advice on health lifestyle behavior with focus on personalized diet and exercise. Participants in the intervention group will undergo the same interview and will also receive text messages in the mobile phone (frequency of the messages as per the wish of the participants) on diabetes education, diet advice and motivation.

Blood tests such as Fasting, 30 and 120 min plasma glucose and insulin, liver enzymes and Lipid Profile will be performed during this visit and at annual visits. During 6 and 18 months we will perform 2 hr post glucose load test. If the value reached ≥ 200 mg/dl we will repeat the test within two weeks time. Our staff will administer questionnaires to assess your quality of life, dietary pattern and levels of physical activity.

3. What are the possible risks / discomforts in this study?

As the programme involves lifestyle modification no possible risk is anticipated. Identification of undetected diabetes or hypertension may cause anxiety or fear, in which case you will receive suitable advice and support from the physician regarding management for these disorders. You will also be refereed to your company's physician should the need be.

When blood samples are taken you may experience some discomfort which will subside in due course.

4. What are the possible benefits of participation?

By ascertaining your risk factors you will understand your future chances of diabetes.

You will receive advice on healthy lifestyle – with individualized counseling on diet and physical activity.

By participating in this study you will have a better understanding of diabetes awareness and ways of prevention.

During assessment there may be chances of detecting diabetes or hypertension which was previously undiagnosed.

You will be followed up for the next two years with annual blood investigations.

5. Your choice of participation?

Participation in this study is entirely voluntary. Refusal to participate will not affect any benefits you are entitled. If you feel that this programme is disturbing / harming you by any means you can withdraw from this study at any point of time.

6. Will your personal information be kept confidential?

During your participation in this study we will collect personal information and information related to your health. India Diabetes Research Foundation will be responsible for managing your data. The data may be shared with other institutions to effectively analyse the study outcomes. Under all circumstances your personal identity will not be disclosed.

7. Will it cost you anything to be in this study?

Your participation in this study will not cost you any money. You will not be charged for any of your blood tests in this programme.

INFORMED CONSENT FORM

Participant ID:

If you have understood the information explained above and willing to participate in this programme, kindly sign the form below:

1. I confirm that I have read and understood the information for the above programme. ☐
2. I understand that my participation in the programme is voluntary and I am free to withdraw at any time. ☐
3. I understand that my identity will not be revealed in any information given to the third parties or published. ☐
4. I agree not to restrict the use of any data or results that arise from this study for scientific purpose(s). ☐
5. I agree to take part in this programme and follow procedures as explained to me. ☐

(A)_____ / _____ / _____

Signature of the Participant | Name of the participant | Date

(B)_____ / _____ / _____

Signature of the Person obtaining consent | Name of the Person obtaining consent | Date

Appendix - 2

Physical activity assessment

improved educational status. The improved economic conditions were reflected in an increase in motorised transport and access to water and electricity. Fewer subjects were engaged in manual work (22.8% in 2003 vs 80% in 1989). All these parameters had contributed to decreased energy expenditure in domestic as well as occupational functions.

It has generally been observed that there has been a remarkable shift in occupational structure in lower income countries from agricultural labour towards employment in manufacturing and services, resulting in a reduction in energy expenditure and a consequent increase in obesity [9].

We had previously noted that the risk of diabetes occurred at a lower BMI threshold (<23 kg/m^2) and central adiposity (waist circumference: men 85 cm; women 80 cm) in Asian Indians [10]. Therefore, the transition in lifestyle occurring in the rural population seemed to produce rapid adverse changes favouring diabetogenesis.

Prevalence of IGT had remained unchanged in the previous 14 years. The adverse environmental conditions could have favoured the conversion of IGT to diabetes, reducing the number of IGT subjects and increasing the number of diabetic subjects. The ratio of IGT:diabetes was approximately 1. Prevalence of IGT was also higher in the younger rural Indian population, as in the urban population [2].

Awareness of the disease had increased with improved education, and access to medical care was also better. Therefore, 60% of the diabetic cases had already been diagnosed. Similar demographic changes owing to modernisation had been reported in several studies [11, 12, 13].

Improvements in living conditions similar to those noted in our study were described in a neighbouring Bangladeshi population [11]. The prevalence of diabetes had increased from 2.23% to 3.8% in a period of 8 years. A prevalence of 6.5% for diabetes and 11.2% for IGT was reported in rural Pakistan in 1999 [12]. O'Dea et al. noted a significant increase in the prevalence of diabetes in Australian Aborigines consequent to regular contact with urbanised areas [13].

The present study showed that diabetes had increased three-fold over a span of 14 years in a rural population in southern India. The present burden of diabetes in developing countries is chiefly associated with the urban population. The lifestyle transition occurring in the rural areas could further add to this emerging epidemic.

Moreover, according to WHO estimates, the number of diabetic patients in India is underestimated considering the current rate of change in the rural population.

Acknowledgements. We thank all staff members who helped in conducting the screening, especially Ms Lalitha Manjula. Secretarial help from Ms A. Bobby is also acknowledged.

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Appendix I. Physical activity scoring system

Categories of occupation

(i) Manual labourers (including masons, carpenters and those who carry loads, and agricultural work, e.g. ploughing and tilling)
(ii) Office jobs or desk work
(iii) Housewives and retired persons
(iv) Persons unable to work

Does your work involve mostly sitting/standing/walking?

Answer yes.

- Minimum score = 1

Does your work involve moderate activity, e.g. brisk walking, fetching water from wells, moderate agricultural work, e.g. sowing seeds, weeding, painting buildings, household work etc?

Answer yes

- Score = 2 (45 min/day)
- Score = 3 (45–240 min/day)
- Score = 4 (240–480 min/day)
- Maximum score = 28 (4×7 days/week)

Does your work involve vigorous manual activity (occupation category 1)?

Answer yes

- Score = 5 (15–60 min/day)
- Score = 6 (60–240 min/day)
- Score = 7 (240–600 min/day)
- Maximum score = 49 (7×7 days/week)

Additional activities: Do you use a cycle or engage in sports activities?

Answer yes

- Score = 1 (45 min/day)
- Score = 2 (45–240 min/day)
- Score = 3 (240–360 min/day)
- Maximum score = 21 (3×7 days/week)

Total = 70

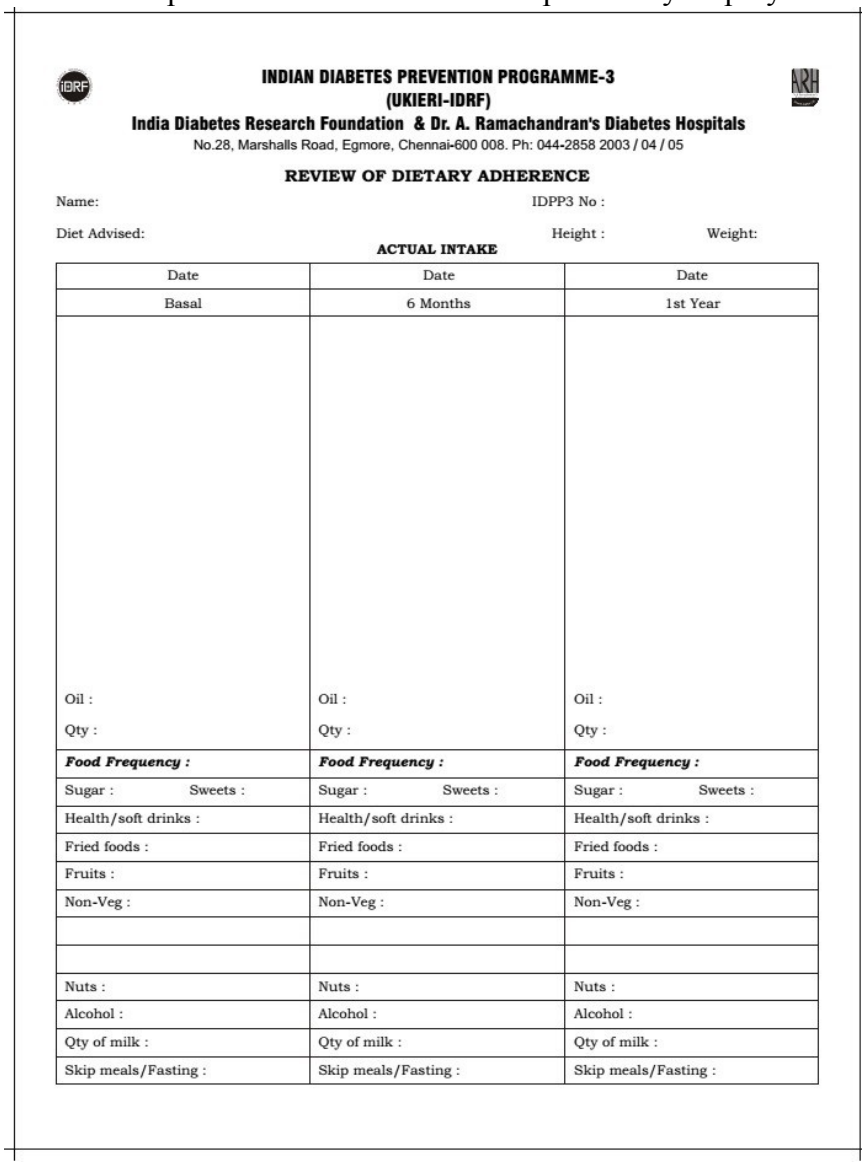
Quartiles of physical activity	Score
1 Sedentary	1–17
2 Light	18–34
3 Moderate	35–51
4 Strenuous	>51

Appendix – 3

Diet Assessment data capture

Habitual nutrient intake was assessed during each visit using 24 hr recall method. Participants were interviewed by a trained nutritionist using a standardised protocol to recall the actual food intake during the previous 24 hr or the previous day. A detailed description of the food list including the estimation of quantities is recorded. This is repeated over several visits and analysed using food composition tables. The total energy intake (kcal) and components of individual food constituents (carbohydrates, proteins and fat (in grams)) consumed by the participants were calculated with an in-house diet analysis program (visual basic programming tool) which uses the National Institute of Nutrition guidelines for India.

Figure-1 shows the template of 24 hr recall method specifically employed for this trial.



INDIAN DIABETES PREVENTION PROGRAMME-3
(UKIERI-IDRF)
India Diabetes Research Foundation & Dr. A. Ramachandran's Diabetes Hospitals
No.28, Marshalls Road, Egmore, Chennai-600 008. Ph: 044-2858 2003 / 04 / 05

REVIEW OF DIETARY ADHERENCE

Name: IDPP3 No :

Diet Advised: Height : Weight:

ACTUAL INTAKE

Date	Date	Date
Basal	6 Months	1st Year
Oil : Qty :	Oil : Qty :	Oil : Qty :
Food Frequency :	***Food Frequency :***	***Food Frequency :***
Sugar : Sweets :	Sugar : Sweets :	Sugar : Sweets :
Health/soft drinks :	Health/soft drinks :	Health/soft drinks :
Fried foods :	Fried foods :	Fried foods :
Fruits :	Fruits :	Fruits :
Non-Veg :	Non-Veg :	Non-Veg :
Nuts :	Nuts :	Nuts :
Alcohol :	Alcohol :	Alcohol :
Qty of milk :	Qty of milk :	Qty of milk :
Skip meals/Fasting :	Skip meals/Fasting :	Skip meals/Fasting :

To simplify the process of data entry and to capture the nutrient intake in a more effective and simplified way the researcher developed an in-house diet inventory programme along with a software technician (using Visual Basic tool). This module comprised of two components:

a. **Food master –** a repository of 217 Indian food items are preloaded and stored in this component. Each food item has its total energy (kcal), carbohydrates (Gms), proteins (Gms), total fats (Gms) and fiber intake (Gms) calculated by the in-house department of nutrition (Dr. A. Ramachandran's diabetes hospitals).

b. **Diet inventory –** the participants' nutrient intake are recorded in this component. It consists of three domains: i) domain-1: wherein the basic information of individuals are recorded along with the visit type viz., baseline, 6 months, 12 months, 18 months and 24 months; ii) domain-2: the information regarding the food item and quantities are recorded; and iii) domin-3: once food items are added, each food item is displayed in the below pane and expands as food items are added further. Finally it gives the cumulative energy intake value along with the individual diet components in Gms.

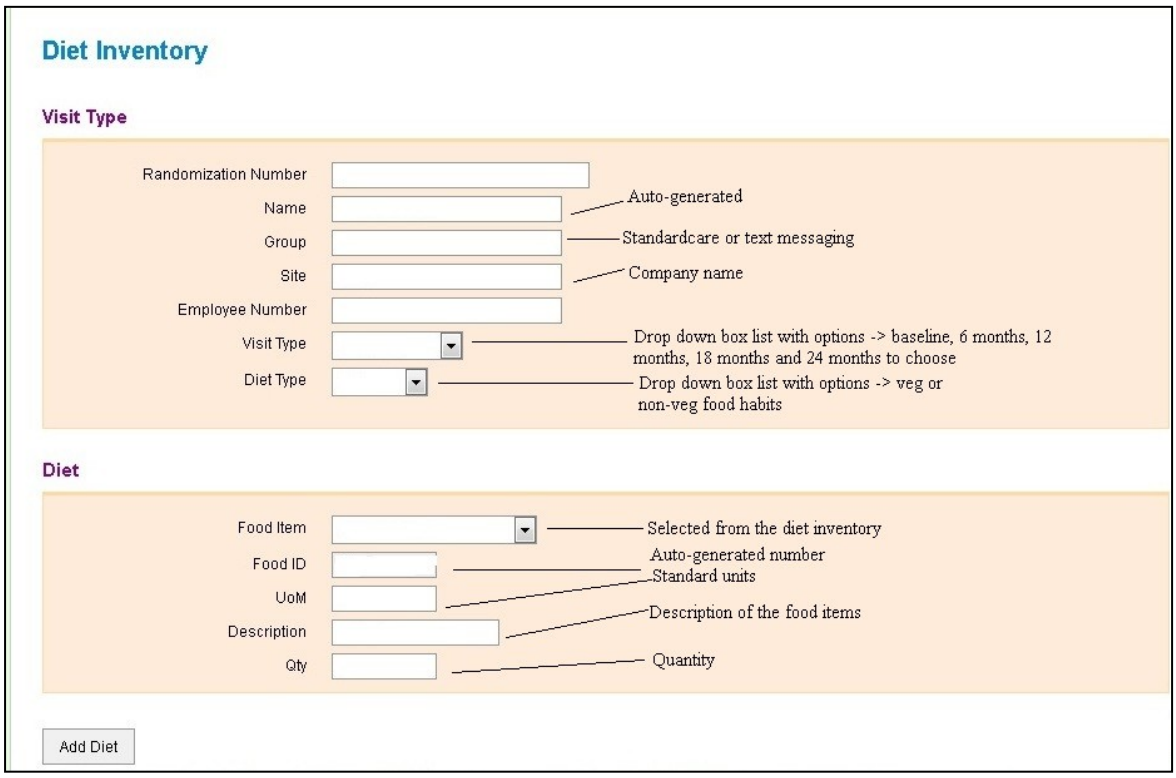
Once the baseline dietary chart for each participant is entered in the database, it automatically reflects the stored data for the subsequent visits. For instance, if we wish to enter the 24 month data, the previously entered data of 18 months will be displayed automatically, which could be modified (quantity of each item added /reduced) to the current observation in a user friendly manner. This is under the assumption that the food pattern of an individual does not change drastically at 6 monthly intervals.

Table: Classification of scores for adherence to diet prescription

Classification	Self-reported adherence
Poor	Not following — <2 days/week (non-adherent)
Fair	Occasional deviation 2–4 days/week (adherent)
Good	Following diet strictly — >5 days/week (adherent)

Dietary adherence (no sugar, ↓ refined carbohydrates, ↓calories, ↑ fibre, ↓ fat) was evaluated every 6 months. Scores were given at each time point to assess the adherence

Figure-2: Scrrenshot of the diet inventory manager developed in-house specific for this trial



Example food frequency data capture information

Food ID	Food Name	UoM	Description	Qty	Kilo Cal	CHO	Protein	Fat	Fibre	Action
1	Rice	1 Cup	25G Raw	4	340	80	8	0	4	Edit / Delete
5	Bread	2 Regular	25G	2	280	40	8	10	0	Edit / Delete
23	Oats	25G	25G	1	95	16	3	2	4	Edit / Delete
26	Dhal	1 Cup	25G	2	170	36	12	2	4.6	Edit / Delete
28	Sambar	1 Cup	25G	1	140	15	7	5	1.5	Edit / Delete
37	Milk (Toned Fat3%)	1 Cup	150 ml	1	90	7	5	5	0	Edit / Delete
55	Rasam	1 Cup	150 ml	1	30	1	0	3	0	Edit / Delete
60	Vegetable Poriyal	1 Cup	75G	1	50	3	1	3	2.2	Edit / Delete
73	Curd	1 Cup	150 ml	2	180	14	10	10	0	Edit / Delete
103	Mixture/Namkin	100G	100G	0.5	240	24	10	11.5	2	Edit / Delete
106	Chappathi with oil	1	25G Raw	4	440	68	12	12	16	Edit / Delete
107	Tea with sugar	1	150ml	4	200	28	8	8	0	Edit / Delete
110	papad	2nos	5g	1	20	1.5	0.5	1.5	0	Edit / Delete
114	Fruit	1medium	100g	1	61	15	0	0	2	Edit / Delete
117	Oil	1tsp	5g	1	45	0	0	5	0	Edit / Delete
					2381	348.5	84.5	78	36.3	

Appendix - 4

SMS Acceptability questionnaire

Sl. No.	Questions	Yes	No
1.	Are you happy with the messages		
2.	Are you happy with the frequency of SMS		
3.	Do you find it easy to understand the SMS		
4.	Do you find it difficult to understand the SMS		
5.	Do you want us to increase the frequency of SMS		
6.	Do you feel the SMS helps to improve your health		
7.	What is your preferred time to receive SMS; morning, afternoon, evening or after 8.00 p.m.		

Given below are a few questions related to the SMS you receive, kindly give your valuable responses and comments to improve our services.

Appendix – 5

Peer reviewed publications arising from the studies in this dissertation:

1. Ramachandran A, Snehalatha C, Ram J, Selvam S, Simon M, Nanditha A, Shetty AS, Godsland IF, Chaturvedi N, Majeed A, Oliver N, Toumazou C, Alberti KG, Johnston DG: Effectiveness of mobile phone messaging in prevention of type 2 diabetes by lifestyle modification in men in India: a prospective, parallel-group, randomised controlled trial. **Lancet Diabetes Endocrinol** 2013;1:191-198

2. Ram J, Nanditha A, Selvam S, Mary S, Samith Shetty A, Snehalatha C, Johnston DG, Ramachandran A: Screening among Male Industrial Workers in India Shows High Prevalence of Impaired Glucose Tolerance, Undetected Diabetes and Cardiovascular Risk Clustering. **J Assoc Physicians India** 2014;62:312-315

3. Ram J, Snehalatha C, Nanditha A, Selvam S, Samith Shetty A, Godsland IF, Johnston DG, Ramachandran A. Hypertriglyceridemic Waist Phenotype as a Simple Predictive Marker of Incident Diabetes in Asian Indian Men With Prediabetes. **Diabet Med** (Manuscript accepted for publication).

4. Nanditha A, Ram J, Snehalatha C, Selvam S, Mary S, Samith Shetty A, Johnston DG, Ramachandran A: Early Improvement Predicts Reduced Risk of Incident Diabetes and Improved Cardiovascular Risk in Prediabetic Asian Indian Men Participating in a 2-Year Lifestyle Intervention Program. **Diabetes Care** (DOI: 10.2337/dc14-0407)

5. Ram J, Selvam S, , Snehalatha C, Nanditha A, Mary S, Samith Shetty AJohnston DG, Ramachandran A: Improvement in Diet Habits, Independent of Physical Activity Helps to Reduce Incident Diabetes among Prediabetic Asian Indian Men. Diabetes Res Clin Practice (accepted).

Poster presentations / conference abstracts from the studies in this dissertation

1. Ram J, Snehalatha C, Nanditha A, Samith Shetty A, Johnston DG, Ramachandran A. Gamma Glutamyl Transferase (GGT) Levels Reflect Insulin Resistance in Subjects with Persistent Impaired Glucose Tolerance. (American Diabetes Association 72nd Scientific Sessions).

2. Ram J, Snehalatha C, Nanditha A, Samith Shetty A, Johnston DG, Ramachandran A. Association of serum leptin levels with surrogate measures of insulin action in Asian Indian Subjects with impaired glucose tolerance. Research Society for the Study of Diabetes in India (2012).