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Effects of the Lysulin™ supplementation on pre-diabetes: A randomized double-blind, placebo-controlled clinical trial

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ABSTRACT

Background

Diabetes mellitus is a leading cause of morbidity and mortality worldwide. Recent studies have demonstrated that nutraceutical products have beneficial effects in diabetes. Present

study aims to investigate whether a product (Lysulin™) containing amino acid Lysine, micronutrient Zinc and Vitamin C will have beneficial effects on glycaemic control and disease progression in pre-diabetes.

Methods

A randomized, double-blind, placebo-controlled clinical trial was conducted for a period of 6 months. Study included two parallel groups (Lysulin™ and placebo). One hundred and ten subjects with pre-diabetes were recruited. Evaluations were done at baseline, 1, 3 and 6 months. Primary outcome was defined as change in glycaemic control measured by HbA1c from baseline. Other outcomes included change in; fasting plasma glucose (FPG), 2-hour OGTT plasma glucose and lipid profile from baseline. Three multiple regression analyses were performed, where change in FPG, 2-hour OGTT, and HbA1c post intervention from baseline respectively were the continuous dependent variable with other independent variables.

Results

One hundred and ten patients were recruited, 50% (n=55) were males and mean age (±SD) was 46.7±9.9 years. During the 6-month follow-up period a significantly higher percentage of participants in Placebo group (25.4%,n=14) developed type 2 diabetes in comparison to Lysulin™ group (7.3%,n=4) (p=0.018) (OR: 4.3). FPG, 2-hour OGTT and HbA1c significantly reduced in the Lysulin™ group only. Observed HbA1c reduction during 6-month follow-up period in Lysulin™ group is 0.5%. Both total cholesterol and LDL cholesterol decreased significantly from baseline only in the Lysulin™ group. In all three regression models the best predictor of respective dependent variable was Lysulin™ treatment.

Conclusions

Lysulin™ improved glycaemic control, with reduced progression to diabetes, in those with pre-diabetes. The treatment also showed a beneficial reduction in total and LDL cholesterol levels. Further studies are required to fully elucidate the mechanisms responsible.

Trial Registration: Sri Lanka Clinical Trials Registry, identifier: SLCTR/2018/022 (http://slctr.lk/trials/1290). Registered on 13th July 2018; Study protocol version 2.0 (23rd March 2018)

Keywords: Lysulin, Pre-diabetes, Lysine, vitamin C, Zinc

BACKGROUND

Diabetes mellitus is a leading cause of morbidity and mortality worldwide [1]. Type 2 diabetes mellitus is the commonest form of the disease [2]. The costs to even provide basic health care for these patients are substantial, overwhelming the existing health care facilities even in the most developed countries. Therefore, preventive measures have a unique place especially in parts of the world where diabetes is becoming increasingly prevalent. The causes of type 2 diabetes are multifactorial, and the diet plays an important role on its incidence, severity and management [3]. Therefore, studies have frequently focused on dietary components beneficial in the prevention and treatment of diabetes. Recent studies have demonstrated that nutraceutical products have beneficial effects in patients by improving glucose and lipid metabolism, antioxidant status and disease progression[4].

Lysulin™ is such a nutraceutical tablet manufactured in the USA which contains a proprietary amount of the essential amino-acid Lysine, micronutrient Zinc and Vitamin C[5]. Lysine is an essential amino acid that plays a major role in calcium absorption, building muscle protein, and the body's production of hormones, enzymes, and antibodies. It has also shown numerous beneficial effects in the treatment of diabetes and/or it's complications in in-vivo animal and human studies [6, 7]. Lysine is known to react with glucose with the glycated amino acid being excreted in urine and it has been shown to markedly attenuate the glucose response to ingested glucose without a change in insulin response in humans [8]. Furthermore, studies have shown that it reduces the formation of glycated proteins in diabetes induced animal models [6].

Zinc is involved in numerous metabolic pathways as a cofactor for more than 300 enzymes [7]. Insulin, which contains a variable number of Zinc atoms, is stored in β-cells of the pancreas and released into the portal venous system at the time of β-cells degranulation. Zinc plays an important role for insulin action, carbohydrate and protein metabolism [10]. It has been long known that diabetes is accompanied by hypozincemia [12] and hyperzincuria [13]. Zinc absorption is also known to be altered in patients with diabetes [9]. Numerous studies have shown that Zinc supplementation improves glycaemic control in patients with type-2 diabetes, with a resultant reduction in HbA1c of around 0.5% in pooled analysis [10]. A recently concluded clinical trial on patients with pre-diabetes demonstrated that Zinc supplementation helps to reduce blood glucose and insulin resistance, while improving β-cell function. Furthermore, disease progression to diabetes was also reduced and beneficial effects of supplementation were also noted on total and LDL cholesterol [11].

Ascorbic acid (vitamin C), an antioxidant vitamin, plays an important role in protecting free radical-induced damage. Previous study has shown decrease in basal vitamin C level in type 2 diabetes [12]. Vitamin C is structurally similar to glucose and can replace it in many chemical

reactions and thus is effective for prevention of nonenzymatic glycosylation of protein [13]. Furthermore, randomized controlled trials have shown that supplementation of Vitamin C reduces blood glucose, serum lipids and improves HbA1c in type 2 diabetes [14]. Hence, we postulate that a product containing Lysine, Zinc and Vitamin C will have beneficial effects on glycaemic control and disease progression in patients with pre-diabetes. The present study aims to evaluate this hypothesis using a phase II/III randomized double-blind controlled clinical trial design.

METHODS

Study setting and design

Reporting of the present study is done according to the CONSORT statement (Consolidated Standards for Reporting Trials) (Supplementary File 1). This randomized (1:1), double-blind, placebo-controlled phase II clinical trial was conducted at the Nawaloka Hospital Research and Education Foundation (NHREF), Nawaloka Hospitals PLC, Colombo, Sri Lanka for a period of 6 months, assessing the effects of daily supplementation of Lysulin™ tablets in patients with pre-diabetes.The study included two parallel groups (treatment group [Lysulin™tablet] and control group [placebo tablet]). The study was approved by the Ethics Review Committee (ERC) of Faculty of Medicine, University of Colombo (EC/18/020) and subsequently registered at the Sri Lanka Clinical Trials Registry (SLCTR/2018/022). Detailed description of the study protocol is described elsewhere and only a summary is presented here[15].

Study population and sampling

One hundred and ten subjects with pre-diabetes were recruited for the study after eligibility screening against inclusion/exclusion criteria. Pre-diabetes was defined as the presence of Fasting Plasma Glucose (FPG) between 100-125mg/dl (Impaired Fasting Glucose [IFG]) or 2-hr Post Oral Glucose Tolerance Test (OGTT) Plasma Glucose between 140-199mg/dl (Impaired Glucose Tolerance [IGT]) or both IFG and IGT or a HbA1c value between 5.7-6.4% [16]. The number of patients required for determination of a 0.5% reduction of HbA1c in the treatment arm, in comparison with the placebo arm at 80% power and 95% confidence interval with a drop-out rate of 20% was 108 patients. Hence, a total of 110 adults with diagnosed pre-diabetes was recruited for the study. Subjects were randomly and equally assigned into two groups (n=55 each) and received either Lysulin™ oral tablet or an identical placebo daily for a period of 6 months.

Inclusion/exclusion criteria are described in detail elsewhere[15]. In summary the inclusion criteria were, a) age between 18-70 years and b) screening test confirming presence of pre-diabetes as defined above. The exclusion criteria were, a) on any other vitamin or mineral supplementations, b) the current use of a weight loss medicine or dietary modification, c) history of diabetes mellitus, d) Presently having acute diseases or other untreated illness requiring treatment, e) impaired hepatic or renal functions, f) patients with any malignancy or any other unrelated chronic illness, g) patients with cardiac, liver or respiratory failure, h) Allergy to any of the constituents of the tablets, i) lactation, pregnancy or unwillingness to use an effective form of birth control for women of child bearing age and h) any condition in the opinion of the primary investigator that would contraindicate the patient's participation.

Study participants satisfying the eligibility criteria mentioned above were informed about the study, its duration and participant responsibilities, and subjects were recruited only after obtaining informed written consent. The patients were randomized according to the method of block randomization with a block size of 4. The population was stratified at randomization based on Age (<30 years and ≥ 30 years) and gender to ensure equal distribution of these variables in the two arms. A computer-generated sequence was used for randomization. The investigators and patients were blind to the treatment allocations. The allocation sequence number generation was done by an independent third party not involved in the trial and was kept in a secure place during the course of the study. Concealment of the randomization sequence was done by using sequentially numbered, opaque sealed envelopes [17]. Eligibility assessment and enrolment was done by one independent investigator, while another investigator was involved in randomization.

The intervention, follow-up and outcomes

The treatment nutritional supplement (Lysulin™) was a tablet containing amino acid Lysine, elemental Zinc and Vitamin C as the active ingredients. The placebo tablet contains inactive ingredients (magnesium stearate and microcrystalline cellulose) and was manufactured to have a similar appearance, shape, weight, taste, color, smell and texture. The manufacturer was responsible for the labeling of the tablet bottles with the code numbers. Recruited subjects received either two tablets of Lysulin™ or placebo three times daily, taken 1 hour before meals for a period of 6 months. Bottles containing a one-month supply of the tablets were given to the patients in the respective groups at each visit. Participants in both groups received uniform advice about diet and physical activity, which are considered to be potential confounder variables affecting glycaemic control.

The study was conducted for a period of 6 months and the evaluations were done as follows; screening (visit 0), 1 month (visit 1), 3 month (visit 2) and 6 month (visit 3). The primary

outcome was defined as change in glycaemic control as measured by HbA1c from baseline.A full list of the secondary outcomes assessed at each time point is described elsewhere[15], these included: change in FPG and OGTT plasma glucose from baseline; development of diabetes during follow-up, as indicated by either FPG >125 mg/dL and/or 2-hour OGTT plasma glucose of >199 mg/dL and/or HbA1c>6.5%, confirmed during follow-up visits [18]; change in lipid profile from baseline (Total cholesterol, LDL, HDL and TAG); change in insulin resistance from baseline where insulin resistance was measured by the Homeostasis Model of Assessment- Insulin Resistance (HOMA-IR) calculations based on fasting blood glucose and fasting serum insulin; change in anthropometric assessment such as body weight, height, Body Mass Index [BMI], waist circumference [WC], hip circumference [HC] and Waist:Hip Ratio [WHR] from baseline and change in systolic (SBP) and diastolic blood pressure (DBP) from baseline. Level of physical activity (International Physical Activity Questionnaire [IPAQ] short form) and dietary intake (validated Food Frequency Questionnaire) was assessed in both groups as they are confounding factors affecting glycaemic control. The following parameters/information was measured/recorded for the safety assessment during follow-up; vital signs, serum creatinine, liver enzymes, serum bilirubin and adverse events. A summary of each outcome (primary, secondary and safety) assessed during follow-up visits is presented in Table 1. Compliance was calculated by pill counting based on the number of tablets returned during each follow-up visit.

Data collection, biochemical analysis and definitions

Data collection during follow-up visits was carried out by a team of trained research assistants. All anthropometric measurements (height, weight, BMI, WC and HC) were made by using standard calibrated equipment and following WHO guidelines. Details of anthropometric and clinical measurements have been described in detail elsewhere[15]. Seated SBP and DBP were measured after a 10-min rest with Omron IA2 digital BP monitors (Omron Healthcare, Singapore). The equipment used for anthropometric measurements were calibrated by the Department of Measurement Units, Standards and Services, Colombo, Sri Lanka [19]. A culturally validated FFQ was used to obtain habitual intake of calorie, macronutrients and micronutrients[20].Physical activity was assessed using the translated and validated short version of the IPAQ (International Physical Activity Questionnaire) administered by an interviewer and are presented as energy expenditure expressed in MET minutes/week (Metabolic Equivalent-Minutes)[21].Biochemical tests were performed in the accredited laboratory of the Nawaloka Hospital PLC, Colombo, Sri Lanka.

Statistical analysis

Parametric and non-parametric statistical tests were applied using the SPSS statistical software (SPSS Inc., Chicago, IL, USA) for the data analysis. FFQ data was analyzed using NutriSurvey 2007 (EBISpro, Germany), nutrient analysis software for Windows, modified for Sri Lankan food items and recipes and is reported as the intake of calories, carbohydrates, fat, proteins and dietary fibers per day. Summary statistics were calculated and presented as mean, standard deviation and proportion by groups. The baseline and end of study characteristics as well as the laboratory findings of the groups were compared using two sample and paired t test and a P value <0.05 was considered significant. Homeostasis Model Assessment (HOMA2) calculator was used to calculate β-cell function (HOMA – β) and insulin resistance (HOMA - IR) based on fasting insulin and plasma glucose[22].Where appropriate, sensitivity analyses were conducted (for example, control for additional covariates; and bootstrapped p values for skewed outcomes). In the case of missing data values, we applied mean imputation and regression imputation where rates are low and consider multiple imputations where they exceed 10%.

A multiple regression analysis was performed, where change in FPG post intervention from baseline was the continuous dependent variable and independent variables were age (continuous), treatment group (0-Placebo, 1-Lysulin™), gender (0-female, 1-male), physical activity (continuous), energy intake (continuous), carbohydrate intake (continuous) and baseline FPG. Similar multiple regression analyses were performed for change in 2-hour OGTT and HbA1c post intervention from baseline as the continuous dependent variable and other independent variables mentioned above.

RESULTS

One hundred and ten patients were recruited for the study and randomized to Lysulin™ and Placebo groups, 50% (n=55) were males and mean age (±SD) was 46.7±9.9 years (range 25-70). Participant completing 1, 3 and 6-months follow-up was 105 (Lysulin™ group – 52 and Placebo group – 53), 94 (Lysulin™ group – 49 and Placebo group – 45) and 83 (Lysulin™ group – 45 and Placebo group – 38) patients respectively. Nine patients (Lysulin™ group – 4 and Placebo group – 5) were lost to follow-up, while the remaining 18 patients discontinued the respective interventions due to development of diabetes as defined above (Lysulin™ group – 4 and Placebo group – 14). Patient enrollment, allocation, follow-up and analysis details are summarized in Figure 1. Baseline characteristics of the study population, including age, clinical, anthropometric and biochemical parameters are presented in Table 2. Only Total cholesterol and daily fat intake were significantly different between the Lysulin™ and Placebo groups at baseline (Table 2).

Glycaemic control and disease progression

During the 6-month follow-up period a significantly higher percentage of participants in the Placebo group (25.4%, n=14) developed type 2 diabetes in comparison to the Lysulin™ group (7.3%, n=4) (p=0.018) (OR: 4.3 [95%CI 1.3-14.2]). The FPG significantly reduced in the Lysulin™ group at 3 months and this reduction was sustained during the 6-month follow-up period, however this was not observed in the Placebo group (Table 3). A similar reduction was observed in the 2-hour OGTT plasma glucose values and HbA1c only in the Lysulin™ group, whereas in the Placebo group they have either remained unchanged or significantly increased (Table 3). The observed HbA1c reduction during the 6-month follow-up period in the Lysulin™ group is 0.5%. Insulin resistance (HOMA-IR) decreased significantly from baseline to6 months in the Lysulin™ group, with significant improvement in β-cell function (HOMA-β) (Table 3). However, this was not observed in the control group.

Blood pressure, anthropometric, clinical and other biochemical parameters

Systolic and diastolic blood pressure remained significantly unchanged from baseline values during follow-up, both in the Lysulin™ and Placebo groups (Table 4). A similar observation was noted for anthropometric parameters (BMI, WC, HC and WHR) in both groups (Table 4). Both total cholesterol and LDL cholesterol decreased significantly from baseline in the Lysulin™ group, and it remained unchanged in the Placebo group (Table 5). HDL cholesterol did not change significantly in both groups. In the Placebo group triglycerides reduced significantly during follow-up, while triglycerides remained unchanged in the Lysulin™ group. The analysis of the food frequency questionnaires at each visit (0-3) did not reveal a significant difference in the energy, carbohydrate, protein, fat and dietary fiber intake between the two groups in an across group comparison (data not shown). Physical activity (total MET minutes/week) was also similar between groups and within groups at baseline, 3 months and 6 months.

Multiple linear regression analysis

The results of the multiple regression analyses are summarized in Table 6. All three regression models were statistically significant with an adjusted R^2 of 0.432 (FPG), 0.511 (OGTT) and 0.329 (HbA1c). Significant predictors of change in FPG from baseline were, age, carbohydrate intake and Lysulin™ treatment (Table 6). Change in 2-hour OGTT plasma glucose was predicted by carbohydrate intake, baseline 2-hour OGTT value and Lysulin™ treatment. Similar results were observed for HbA1c, where carbohydrate intake and Lysulin™ treatment were the significant predictors of HbA1c.In all three regression models the best predictor of the respective dependent variable was Lysulin™ treatment (Table 6).

Adverse effects, safety and compliance

There were no serious adverse effects noted and none of the subjects were hospitalized due to adverse effects during the 6 months follow up period. No hypoglycemic episodes were reported in the study participants. Biochemical assessments evaluating potential target organ toxicity (liver enzymes, serum bilirubin and serum creatinine) remained normal throughout the study period (data not shown). None of the patients experienced any form of hypersensitivity during the study (immediate and/or delayed). Drug compliance (%) of patients was evaluated by pill counting. The mean % compliance (±SD) during the 1st month, 3rd month and 6th month in the Lysulin™ group was 90.8±19.2, 91.2±10.6, 92.5±10.1 and 90.6±19.1 respectively. A similar high compliance was noted in the Placebo group (91.9±19.7, 90.1±10.6, 91.7±11.5 and 93.5±6.1 respectively). There was no significant difference between the mean % compliances of the two groups.

DISCUSSION

In this first randomized controlled clinical trial evaluating the effects of the nutraceutical Lysulin™ containing amino acid Lysine, Zinc and Vitamin C in pre-diabetes, we observed a beneficial effect on glycaemic control with reduced blood glucose (both fasting and OGTT) and HbA1c, with reduced progression to diabetes in the treatment group compared to placebo. Furthermore, Lysulin™ treatment was a significant predictor of glycaemic control in regression models, adjusting for confounder variables. In addition, Lysulin™ reduced insulin resistance, whilst improving ß-cell function in those with pre-diabetes. The treatment group also showed a beneficial reduction in total and LDL cholesterol level, in comparison to placebo. Hence, it is evident that a combination nutraceutical with the above ingredients are likely to be of benefit in pre-diabetes. All of the ingredients independently have been previously shown to have beneficial effects on glycaemic control in-vivo animal and human studies. However, the present study is the first to evaluate the effects of the combination in pre-diabetes and its effect on disease progression to diabetes.

Zinc supplementation alone has been shown to reduce the progression of disease in pre-diabetes, with added beneficial effects on total and LDL cholesterol[23]. However, in this previous study the magnitude of HbA1c reduction has not been evaluated, although similar rates to the present study were observed in relation to disease progression. Our results demonstrate that LysulinTM reduces HbA1c by 0.5%. The HbA1c reduction obtained with current oral anti-diabetic therapy is between 0.5-2.0% [24]. However, these medications are currently not recommended for the treatment of pre-diabetes, which is mainly managed by lifestyle measures at present. The fact that LysulinTM also demonstrates a comparable HbA1c reduction, without any hypoglycemic adverse effects indicates that it may be considered as a

nutraceutical intervention in pre-diabetes, in addition to lifestyle measure. Molecular level studies have shown that Zinc plays an important role in β-cell function, insulin action, glucose homeostasis and the pathogenesis of diabetes and its complications[25].All these factors are likely to contribute towards beneficial effect observed in the present study.

LysulinTMalso contains Vitamin C and the amino acid Lysine, both which have shown beneficial effects on glycaemic control in diabetes. However, to date the present study is the first to evaluate Vitamin C and Lysine in pre-diabetes. A recent meta-analysis has shown that Vitamin C causes a significant improvement of glucose concentration in patients with diabetes, older participants and in studies with longer duration [26]. Previous epidemiological analyses have shown that a higher antioxidant capacity was linked to reduced insulin resistance and better glucose control [27]. This is an additional advantage that is conferred by Vitamin C, which has strong antioxidant properties. The observed improvement in insulin resistance and β-cell function in the present study could be due to the antioxidant properties of both Zinc and Vitamin C. Lysine is known to react with glucose with the glycated amino acid being excreted in urine and it has been shown to markedly attenuate the glucose response to ingested glucose without a change in insulin response in humans [8]. The observed reduction in 2-hour OGTT blood glucose with LysulinTM is likely to be contributed by the amino acid Lysine, via the above mechanism.

Glycated proteins (AGEs) are known to be involved in the pathogenesis of several chronic diabetes complications, including nephropathy leading to chronic kidney disease, neuropathy, and retinopathy, as well as in other macrovascular complications [28-30].Once set in motion, glycation-promoting mechanisms may stimulate ongoing AGE production and target tissue stresses that reduce insulin responsiveness, a cardinal feature of both pre-diabetes and diabetes [31].Studies have shown that all three ingredients of Lysulin™, namely amino acid Lysine [6], Vitamin C [13] and Zinc [32]reduces the formation of glycated proteins. This is likely to contribute towards the observed improvement in insulin resistance and β-cell function with Lysulin™. However, these results need to be replicated in studies of longer duration to fully understand the place of LysulinTM in the formation of AGEs and its impact on disease progression in pre-diabetes, as well as diabetes complications.

The present study has several notable strengths. These include 1) assessment of confounder variables affecting Glycaemic control, including physical activity, diet and anthropometric parameters, 2) high compliance rate noted in both study groups and 3) appraisal of adverse effects and toxicity of intermediate-term nutraceutical supplementation. However, a notable limitation is the inadequate sample size (lack of power) to accurately estimate the effect on disease progression. Furthermore, in the absence of original research studies, sample size was estimated based on the assumption that oral Lysulin™ supplementation results in the

reduction of HbA1c similar to oral anti-diabetic agents. Hence, further larger studies are required to further validate these findings and explore mechanisms of action.

CONCLUSIONS

Lysulin™ containing amino acid Lysine, Zinc and Vitamin C, improved glycaemic control, with reduced progression to diabetes, and improved ß-cell function in those with pre-diabetes. The treatment also showed a beneficial reduction in total and LDL cholesterol levels. These results need to be replicated in larger randomized controlled trials and further studies are also required to fully elucidate the mechanisms responsible.

DATA AVAILABILITY

Data can be made on request from the corresponding author

CONFLICTS OF INTEREST

The author(s) declare that they have no competing interests.

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TABLES

Table 1: A brief study schedule at every visit

	Visit 0 (baseline visit)	Visit 1 (1 month)	Visit 2 (3 month)	Visit 3 (6 month)
Informed consent form	●	--	--	--
Recording demographic data	●	--	--	--
Medical history taking	●	●	●	●
Physical examination[1]	●	●	●	●
Fasting Plasma Glucose	●	●	●	●
2-hour OGTT plasma glucose	●	●	●	●
HbA1c	●	--	●	●
Serum insulin	●	--	--	●
Lipid profile[2]	●	--	●	●
Liver function[3]	●	●	●	●
Renal function[4]	●	●	●	●
Blood pressure	●	●	●	●

Food Frequency Questionnaire	●	--	--	●
Physical Activity	●	●	●	●

[1]body weight, height, waist circumference and hip circumference; [2]total cholesterol, triglyceride, LDL cholesterol and HDL cholesterol; [3]AST, ALT and total bilirubin; [4]creatinine

Table 2: Baseline characteristics of the Lysulin™ and Placebo groups

	Mean (±SD)		
	Lysulin™ Group (n=55)	Placebo Group (n=100)	p value
Age (years)	46.2 (±9.6)	44.5 (±10.6)	NS
Blood Pressure			
Systolic blood pressure (mmHg)	129.3 (±17.5)	136.0 (±19.0)	NS
Diastolic blood pressure (mmHg)	77.3 (±10.6)	81.5 (±9.4)	NS
Anthropometric parameters			
Body mass index (kg/m^2)	26.1 (±3.4)	24.6 (±3.7)	NS
Waist circumference (cm)	86.9 (±8.6)	87.5 (±7.9)	NS
Hip circumference (cm)	94.1 (±6.7)	95.5 (±6.4)	NS
Waist to Hip ratio	0.92 (±0.06)	0.92 (±0.07)	NS
Physical Activity	830 (±1123)	782 (±1318)	NS
Dietary Intake			
Total energy (kcal/day)	1658.1 (±459.5)	1771.1 (±420.3)	NS
Carbohydrate (g/day)	353.1 (±98.5)	392.4 (±96.9)	NS
Protein (g/day)	42.2 (±17.4)	45.2 (±16.1)	NS
Fat (g/day)	46.9 (±14.2)	55.9 (±18.1)	0.004
Dietary fiber (g/day)	15.6 (±7.9)	16.9 (±8.4)	NS
Biochemical parameters			
Fasting Plasma Glucose (mg/dl)	108.9 (±13.7)	107.3 (±13.3)	NS
2hr OGTT Plasma Glucose (mg/dl)	142.0 (±15.7)	149.2 (±15.5)	NS
HbA1C (%)	5.9 (±0.5)	6.1 (±0.7)	NS
Serum insulin (μIU/l)	11.8 (±6.0)	13.1 (±9.9)	NS
Insulin resistance	1.3 (±0.5)	1.1 (±0.5)	NS
β-cell function (%)	52.9 (±19.2)	51.1 (±22.7)	NS
Total cholesterol (mg/dl)	199.1 (±32.5)	176.0 (±31.9)	<0.001
LDL cholesterol (mg/dl)	129.6 (±38.9)	135.3 (±32.8)	NS
HDL cholesterol (mg/dl)	46.1 (±11.5)	53.4 (±19.9)	NS
Triglycerides (mg/dl)	130.5 (±54.4)	126.2 (±51.0)	NS
Alanine aminotransferase (U/l)	23.4 (±10.0)	24.1 (±11.7)	NS
Aspartate aminotransferase (U/l)	29.4 (±7.9)	27.9 (±11.8)	NS
Serum bilirubin (mg/dl)	0.6 (±0.4)	0.6 (±0.3)	NS
Serum creatinine (mg/dl)	0.8 (±0.2)	0.7 (±0.2)	NS

HDL – High Density Lipoprotein; LDL – Low Density Lipoprotein; NS – Not Significant; OGTT – Oral Glucose Tolerance Test; SD – Standard Deviation

Table 3: Glycaemic control, insulin resistance and β-cell function in the Lysulin™ and Placebo groups

	Mean±SD			
	Visit 0 (Baseline) (n=110)	Visit 1 (1 month) (n=105)	Visit 2 (3 months) (n=94)	Visit 3 (6 months) (n=83)
FPG (mg/dl)				
Lysulin™	108.9 (±13.7)[#‡]	109.9 (±11.5)	97.2 (±9.8)[#]	95.2 (±8.7)[‡]
Placebo	107.3 (±13.3)	111.1 (±10.4)	105.2 (±9.3)	109.9 (±16.0)
2hr OGTT PG (mg/dl)				
Lysulin™	142.0 (±15.7)[#‡]	142.3 (±10.5)	134.9 (±18.8)[#]	136.0 (±11.2)[‡]
Placebo	149.2 (±15.5)[#]	151.6 (±19.9)	162.8 (±12.5)[#]	155.2 (±16.9)
HbA1c (%)				
Lysulin™	5.9 (±0.5)[#‡]	NM	5.5 (±0.5)[#]	5.4 (±0.3)[‡]
Placebo	6.1 (±0.7)[#]	NM	6.4 (±0.6)[#]	6.3 (±0.6)
Insulin resistance				
Lysulin™	1.3 (±0.5)[#]	NM	NM	1.0 (±0.4)[#]
Placebo	1.1 (±0.5)	NM	NM	1.3 (±0.7)
β-cell function (%)				

Lysulin™	52.9 (±19.2)[#]	NM	NM	71.9 (±10.5)[#]
Placebo	51.1 (±22.7)	NM	NM	49.6 (±15.0)

FPG – Fasting Plasma Glucose; NM – Not Measured; OGTT – Oral Glucose Tolerance Test; SD – Standard Deviation; [*#‡†]values in the same row with similar symbols are significantly different from each other

Table 4: Changes in blood pressure and anthropometric parameters

	Mean±SD			
	Visit 0 (Baseline) (n=110)	Visit 1 (1 month) (n=105)	Visit 2 (3 months) (n=94)	Visit 3 (6 months) (n=83)
Systolic BP (mmHg)				
Lysulin™	129.3 (±17.5)	132.1 (±15.1)	127.3 (±17.2)	135.3 (±19.5)
Placebo	136.0 (±19.0)	129.9 (±17.1)	135.2 (±19.9)	134.5 (±18.9)
Diastolic BP (mmHg)				
Lysulin™	77.3 (±10.6)`	75.1 (±9.9)	81.9 (±13.9)	80.1 (±8.1)
Placebo	81.5 (±9.4)	79.9 (±10.4)	85.0 (±10.1)	79.5 (±9.9)
Body mass index (kg/m^2)				
Lysulin™	26.1 (±3.4)	25.9 (±4.1)	24.8 (±5.1)	25.1 (±3.8)
Placebo	24.6 (±3.7)	25.4 (±4.9)	25.2 (±4.9)	24.5 (±4.6)
Waist circumference (cm)				
Lysulin™	86.9 (±8.6)	87.6 (±9.1)	86.3 (±8.7)	88.2 (±9.1)
Placebo	87.5 (±7.9)	86.4 (±8.1)	86.1 (±9.5)	87.0 (±8.9)
Hip circumference (cm)				

Lysulin™	94.1 (±6.7)	95.0 (±7.2)	96.1 (±9.5)	93.8 (±8.5)
Placebo	95.5 (±6.4)	93.1 (±9.9)	95.7 (±9.6)	96.7 (±6.9)
Waist to Hip ratio				
Lysulin™	0.92 (±0.06)	0.92 (±0.05)	0.90 (±0.06)	0.94 (±0.05)
Placebo	0.92 (±0.07)	0.93 (±0.07)	0.89 (±0.08)	0.90 (±0.06)

BP – Blood Pressure; SD – Standard Deviation

Table 5: Changes in Serum cholesterol and triglycerides

	Mean±SD			
	Visit 0 (Baseline) (n=110)	Visit 1 (1 month) (n=105)	Visit 2 (3 months) (n=94)	Visit 3 (6 months) (n=83)
Total cholesterol (mg/dl)				
Lysulin™	199.1 (±32.5)[#‡]	NM	184.0 (±33.2)[#]	172.1 (±29.1)[‡]
Placebo	176.0 (±31.9)	NM	182.9 (±30.1)	186.9 (±23.9)
LDL cholesterol (mg/dl)				
Lysulin™	129.6 (±38.9)[#‡]	NM	105.9 (±20.1)[#]	117.5 (±16.9)[‡]
Placebo	135.3 (±32.8)	NM	130.2 (±23.8)	128.9 (±38.1)
HDL cholesterol (mg/dl)				
Lysulin™	46.1 (±11.5)	NM	45.2 (±9.1)	44.9 (±8.9)
Placebo	53.4 (±19.9)	NM	56.1 (±15.2)	50.1 (±19.1)
Triglycerides (mg/dl)				
Lysulin™	130.5 (±54.4)	NM	129.5 (±28.2)	118.2 (±39.9)
Placebo	126.2 (±51.0)[#‡]	NM	107.1 (±33.9)[#]	108.5 (±21.9)[‡]

HDL – High Density Lipoprotein; LDL – Low Density Lipoprotein; NM – Not Measured; SD – Standard Deviation

Table 6: Results of the multiple regression analyses

	FPG		2hr-OGTT		HbA1c
	Un-standardized coefficients (95% CI)	P value	Un-standardized coefficients (95% CI)	P value	Un-standardized coefficients (95% CI)
Age	0.75 (0.44, 1.06)	0.012	2.01 (-0.99, 3.02)	NS	-0.20 (-1.46, 1.07)
Male gender	-0.45 (-2.39, 1.90)	NS	-6.95 (-16.45, 2.55)	NS	-0.26 (-1.41, 0.89)
Lysulin™ group	-2.55 (-3.85, -1.25)	<0.001	-3.05 (-5.73, -0.32)	0.027	-3.10 (-4.90,-1.30)
Baseline value*	0.31 (-0.11, 0.77)	NS	0.25 (0.08, 0.42)	0.042	0.14 (-0.02, 0.30)
Physical activity	0.001 (-0.002, 0.002)	NS	−0.01 (−0.04, 0.02)	NS	−0.03 (−0.17, 0.11)
Energy intake	0.10 (-0.09, 0.29)	NS	0.15(-0.03, 0.33)	NS	0.17(-0.05, 0.39)
Carbohydrate intake	1.53 (1.23 1.83)	0.023	-1.82 (-2.86, -0.78)	0.001	-1.36 (- 2.57, -0.14)

CI – Confidence Interval; FPG – Fasting Plasma Glucose; NS – Not Significant; OGTT – Oral Glucose Tolerance Test; * Baseline FPG, OGTT and HbA1c in the respective regression models

FIGURES

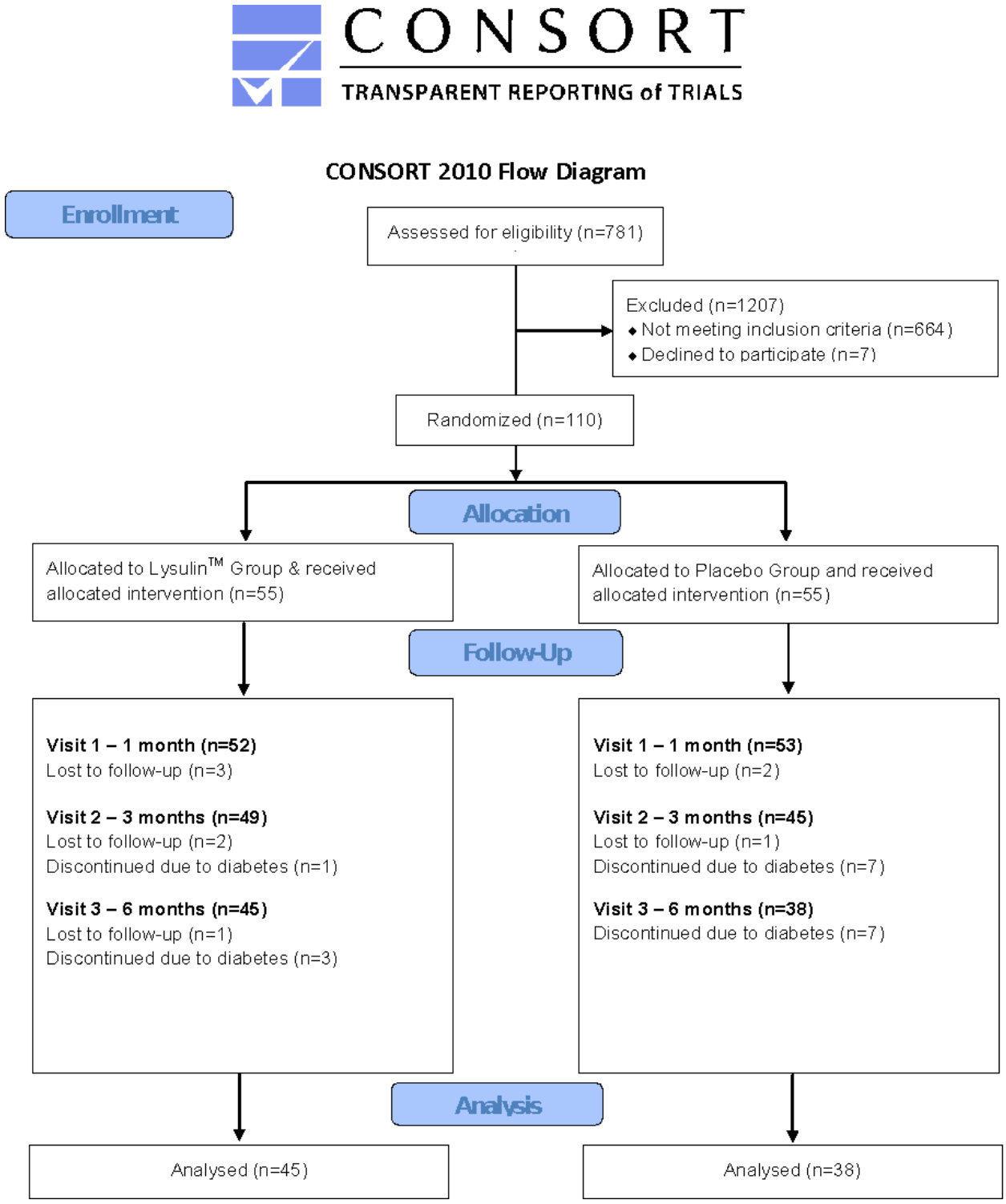

Figure 1: Patient enrollment, allocation, follow-up and analysis