Chapter 4

Experimental studies

MULTI-CENTRIC TRIAL TO ASSESS THE EFFICACY OF *YOGA* ON FASTING AND POST-PRANDIAL BLOOD GLUCOSE AMONG T2DM PATIENTS

4. Introduction

Several research articles, systematic reviews and Meta-analysis have shown the benefits of *Yoga* (chapter 3 of this thesis) on various parameters in urban population in selected groups under controlled conditions. There are very few studies in rural population and few or none in Indian rural population that have looked at the efficacy of *Yoga*. Also, there are no multi-center studies in rural Indian population. Application of the integrated *Yoga* program as a community program as camps has become popular both in India and USA to promote awareness and prevent the rapidly increasing incidence of T2DM. The present study highlights the effort of co-ordination and monitoring at community-based camps with 3 months follow up.

4.1. Aims and objectives

4.1.1. Aim

To assess the impact of 3 months of Integrated *Yoga* (IY) on fasting and post-prandial blood glucose level among patients with T2DM.

4.1.2. Objectives

1. To study the effect of 3 months of *Yoga* -based lifestyle (IAYT) intervention on glucose homeostasis, in type 2 diabetes mellitus.
2. To compare the effect of 3 months of *Yoga* intervention with a control group in a two group multi-centric prospective cluster randomized control trial
3. To study the effect of *Yoga* in urban and rural communities of 6 districts of south India on blood glucose levels.

4.1.3. Hypothesis

Three months of *Yoga* intervention brings reduction in fasting and post-prandial blood glucose levels in Type 2 diabetes mellitus patients.

4.1.4. Null hypothesis

Three months of *Yoga* intervention does not reduce fasting and post-prandial blood glucose levels in Type 2 diabetes mellitus patients.

4.2. Design of the study

Prospective two group multicenter cluster randomized wait list control interventional trial.

4.3. Methodology

4.3.1. Study participants

A house to house survey was conducted between March 2017 to June 2017 in six districts of south India on 1024 individuals from two urban and two rural areas per district of *Andra Prades*h and *Kerala* state to screen for eligibility criteria. Six districts were randomly divided into 2 groups, for either integrated *Yoga* (IY) intervention or for control (3 for each IY and control). A total of 320 participants fulfilled the study criteria; of these, a total of 251 participants, 137 in the *Yoga* group and 114 in the control group who completed the study. Five participants from the *Yoga* group and four from the control group did not present for PPBG during follow up assessments; hence they were excluded from the analysis. Anti-Diabetes medication was kept constant through the entire study period.

4.3.2. Inclusion criteria and Exclusion criteria

4.3.2.1. Inclusion criteria

- Participants with T2DM, between the age range 30-60 years
- Both male and female participants willing to be part of the study were only considered.

4.3.2.2. Exclusion criteria

- Participants with very poor control of diabetes (FBG > 250 mg/dl) or
- Participants with very poorly controlled hypertension (BP systolic> 180mmof Hg and/or diastolic >110mm of Hg)
- Participants taking insulin
- Participants with renal complications of diabetes with creatinine > 3 mg/dl,
- Participants with eye complications of diabetes retinopathy with recent history of laser treatment or advised laser treatment or uncontrolled glaucoma
- Participants with cardiac complications of diabetes, coronary artery disease on medication or history of /or planned for bypass grafting or angioplasty

- Participants who had any other major surgery in the past one year
- Participants on anti-psychotic or steroid medication
- Participants who had previous exposure to any form of *Yoga* in the past one year.

4.3.3. Ethical consideration

The study was approved by the Institutional Ethical Committee of Swami Vivekananda *Yoga* Anusandhana Samsthana (S-VYASA-deemed to be University), Bangalore, India.

Informed consent form was duly signed by all participants before starting the intervention.

4.3.4. Assessments

Participants in both groups were assessed for Fasting and post-prandial blood glucose level by an authenticated NABL accredited laboratory in all venues using the hexokinase method.

4.3.4.1. Fasting blood Glucose (FBG) level

Fasting blood glucose was measured from a blood sample collected early in the morning (between 6-7am) on an empty stomach for all the participants

4.3.4.2.Postprandial blood glucose (PPBG) level

Blood samples were collected from participants for PPBG assessment 2 hours after the break-fast and regular anti-diabetes medication were administered.

4.3.5. Intervention

Participants in IY group received one hour of IY intervention (See Table 7 below) every day, 6 days a week for a period of 3 months. All the practices mentioned in the *Yoga* module have been used in the previous research studies and were found effective in T2DM.

Table- 7: List of 60-minute module of Specific *Yoga* protocol for T2DM used in the study in all centers			
Number	**Name**	**Posture**	**Practices**
1.	Breathing practices (5 minutes)	Standing	Hands Stretch Breathing
		Sitting	Rabbit breathing Tiger Stretch Breathing
2.	Loosening practices *Śithilikaraṇa vyāyāma* (5 minutes)	Standing	*Pādahastāsana* *Ardhacakrāsana vyāyāma*
			Trikoṇāsana vyāyāma
			Kaṭi-parivrattāsana (Spinal Twist)
		Sitting	*Cakkicālana*
			Bhujaṅgāsana
		Supine	*Pavanamuktāsana kriyā*
		Prone	*Dhanurāsana Swing*
3.	Relaxation (5 minutes)		Instant Relaxation Technique
4.	*Sūrya namaskāra* (5 minutes)		12 steps
5.	Asanas (10 minutes each)	Standing	*Ardhakaṭicakrāsana*
			Parivrattatrikoṇāsana
		Sitting	*Vakrāsana*
			Ardhamatsyendrasana
		Prone	*Bhujaṅgāsana* *Dhanurāsana*
		Supine	*Pavanamuktāsana* *Matsyāsana*
	Relaxation (10 minutes)		Deep Relaxation Technique
6.	*Kriyā*		*Kapālabhāti* *Vamanadhauti* (Once a week)
7.	*Prāṇāyāma* (10 minutes)		*Nāṛīśuddhi*
			Bhramarī prāṇāyāma
			`Uccāraṇa (Om chanting)
8.	Meditation (20 minutes)		Cyclic Meditation

4.4. Data analysis

The data was analyzed using SPSS version 20 (Chicago, 2016). Data was presented as mean and standard deviation. The paired sample test was used within the group for comparison; between groups comparison was performed using independent samples test. P value < 0.05 was considered as statistically significant change.

4.5. Results

4.5.1. Demographic details

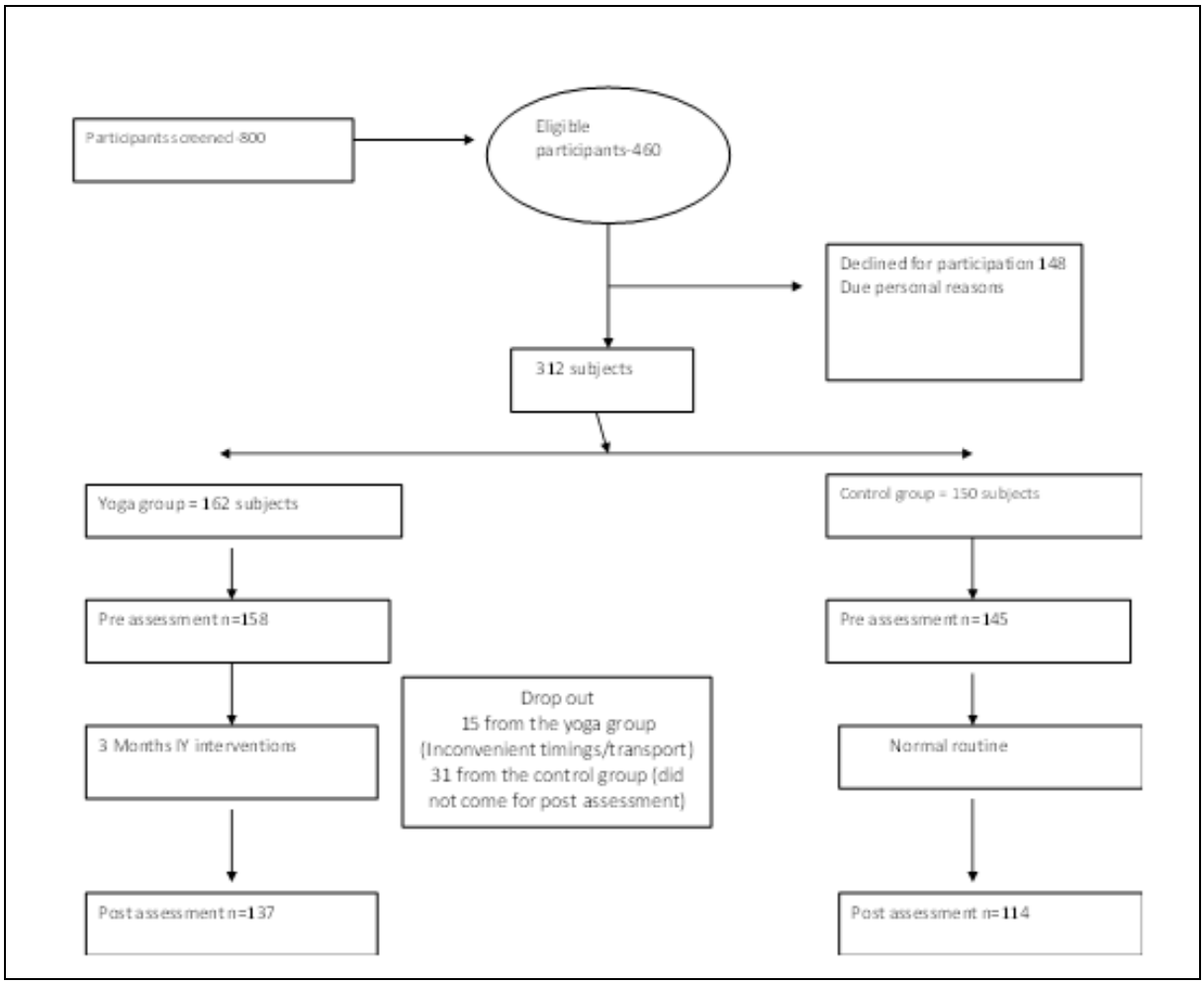
A total of 312 (162 in the *Yoga* group and 150 in the control group) participants who satisfied the selection criteria completed the study. Average age of the participants in the *Yoga* group was 44.15±9.13 years and in the control group it was 46.51±12.93 years. There was no significant difference between the group in term of gender distribution and mean age (See table 8).

Table 8: Demographic details of the participants in the *Yoga* group and control group

Variables	***Yoga* (n=162)**	**Non-*Yoga* group (n=150)**
Gender distribution	Female=88, males =74	Female=82, males= 68
Age (yrs) mean ±SD	44.15±9.13	46.51±12.93

Fig 9- Study1- Consort diagram of multicenter randomized control trial protocol. Out of 800 participants who were screened 460 satisfied the selection criteria and 312 consented to participate in the study, 162 in *Yoga* and 150 in control.

Fig 9: Study Protocol



There were 162 participants in IY group and 150 participants in control group who completed pre-assessments. five patients in the *Yoga* group and 4 participants in the control group did not come for PPBG assessments hence they were excluded from the study;150 participants in the *Yoga* group and 145 in the control group had pre-assessment and were followed up for 3 months. Anti-diabetes medication was kept constant through the entire study.

Final analysis was done on a total of 251 participants data, 137 in the *Yoga* group and 114 in the control group who completed the study. Ten participants from the *Yoga* group discontinued the practice after 2 weeks of the intervention due to problems of transport and inconvenient practice timings; 3 participants had their job transferred; 31 participants from the control group did not complete the post assessments due to personal problems. Thus, the dropout rate was 15 % (44/295).

Within group changes

In *Yoga* group, a significant decrease in both FBG ($p<0.001$, -32.68%) and PPBG ($p<0.001$, -34.77%) was observed after three months of IY practice compared to baseline. The control groups showed no significant ($p>0.01$) improvement in FBG (-2.7% reduction) and PPBG (0.88% reduction) after three months compared to baseline.

Between groups

Post-intervention FBG and PPBG comparison between the groups showed significant difference ($p<0.001$) suggestive of significantly lower FBG and PPBG in *Yoga* group compared to control group.

Table 9: Pre-post changes in mean and SD across the groups

Variables	***Yoga* group (n=137)**			**Control group (n=114)**			**Between group Comparison**
	Baseline	**Post**	**Mean diff**	**Baseline**	**Post**	**Mean df**	
FBG	142.99± 32.20	96.26 ± 11.92**[a]	-32.68	137.62 ± 34.33	133.59 ± 50.86	-2.94%	<0.001[b]
PPBG	203.65 ± 45.92	132.83 ± 22.24**[a]	-34.77	190.97 ± 53.83	189.29 ± 78.11	-0.88%	<0.001[b]
FBG-Fasting blood glucose level; PPBG-Post-prandial blood glucose level. **-Statistical significance at 0.001 level; [a] – Paired sample t test, [b] - Independent t test							

Figure 10 study1: pre-post changes in

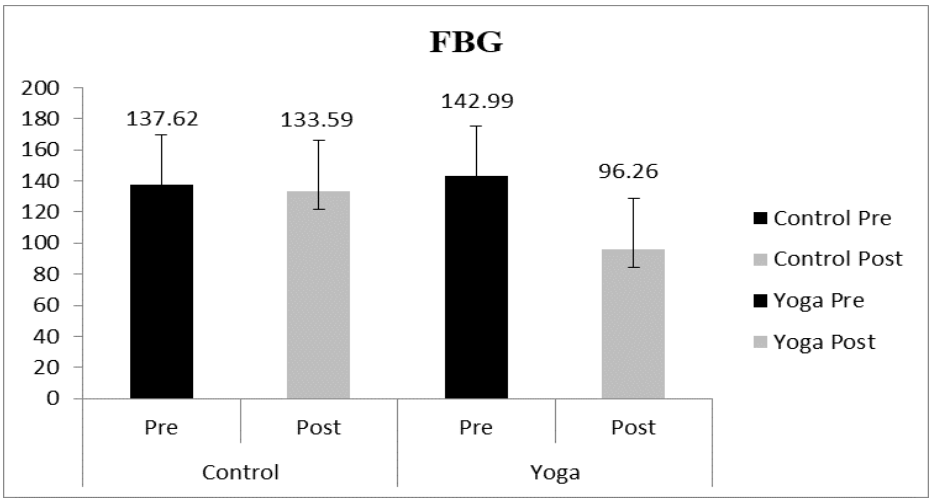
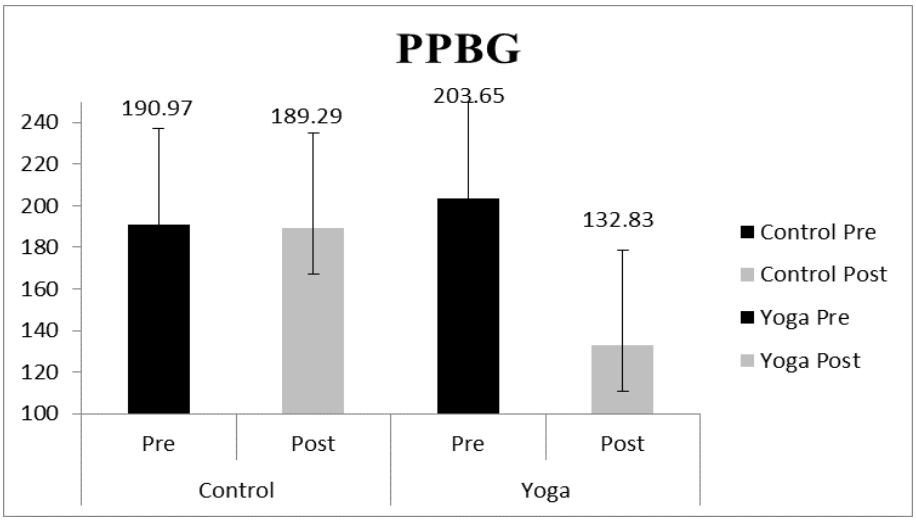


Figure 11 study1 - multicenter study - Pre-post changes in PPBG

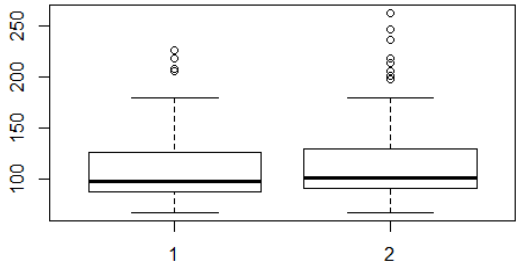


4.5.2. Reduction in standard deviation after *Yoga*

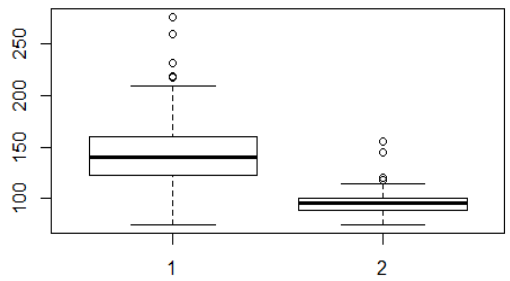
The following graphical representation (box and plot diagram) showing changes in the mean and standard deviation in the *Yoga* and the control group after 3 months. Graphs show marked decrease in SD values of FBG and PPBG in *Yoga* group as compared to control group which indicates normalizing effect of *Yoga* moving towards better homeostasis /balance.

Pre-post changes in FBG:

Study 1- Multicenter trial - Pre post comparison of changes in standard deviation SD in the experimental and control groups



Control



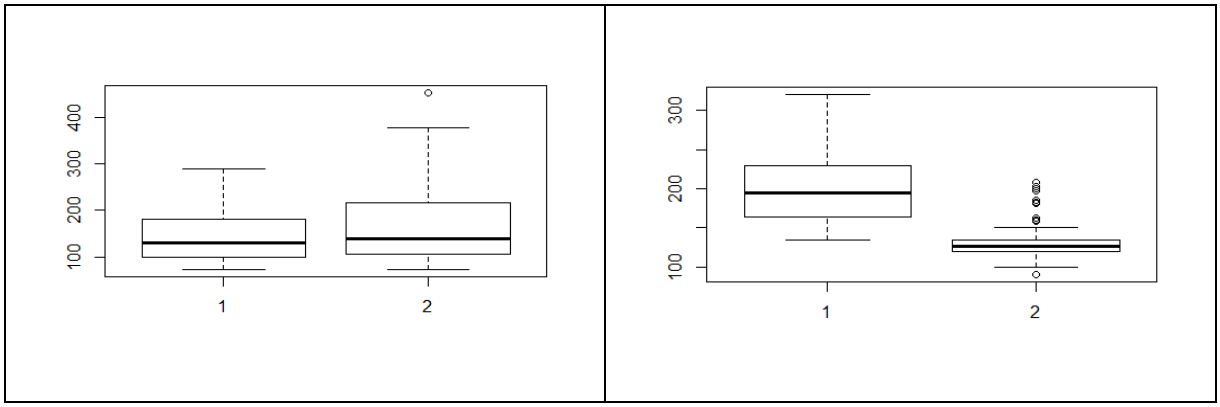
Yoga

Fig 12 Pre (1) – post (2) changes in FBG in the two groups showing reduction in SD and shift in mean values

Pre-post changes in PPBG in two groups

Study 1- multicenter trial- Pre-post comparison of changes in standard deviation SD the experimental and control groups

Fig 13: Pre (1)-post (2) changes in PPBG in the two groups showing reduction in SD and shift in mean values



Control *Yoga*

Fig 12 & Fig 13 shows the changes after 3 months in the number of participants who continued to be in normal range of FBG and PPBG. This was significantly ($p<0.05$ chi square test) higher in *Yoga* than the non- *Yoga* group.

Table 10 below shows the changes after 3 months in the number of participants who continued to be in high range of FBG and PPBG. This was significantly ($p<0.05$ chi squared test) lower in *Yoga* than the control group.

Table 10 - study 1- multicenter trial- Number of participants with pathologic values for FBG and PPBG before and after intervention

Variables	***YOGA***		**Control group**		**Sig between groups chi^2**
	Pre	Post	Pre	Post	**P***
FBG	96	58	60	66	<0.05
PPBG	98	40	56	65	<0.05

*chi square test. Significantly lesser number of pts with T2DM were in pathological range of glucose values in *Yoga* than control group after 3 months

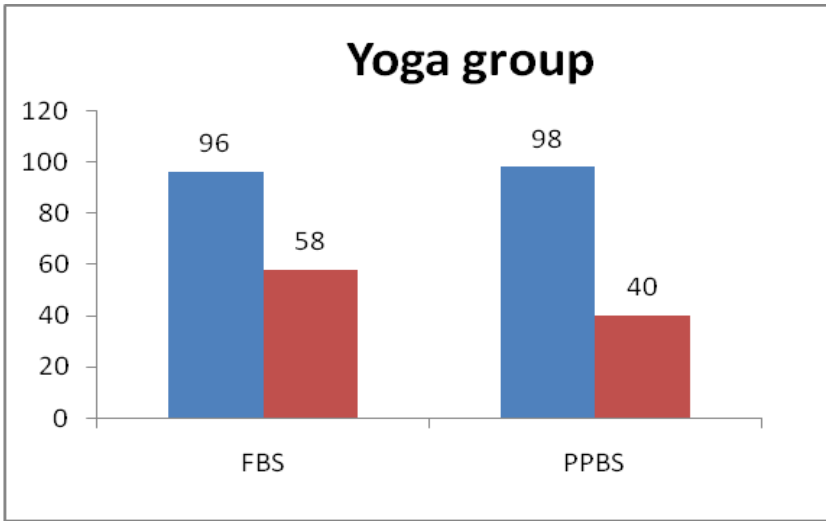
Table 11 and Fig 14 & 15 shows the changes after 3 months in the number of participants who continued to be in normal range of FBG and PPBG. This was significantly ($p<0.05$).

Table 11 - study 1- multicenter trial- Number of participants with normal values for FBG and PPBG before and after intervention

Variables	*Yoga*		Control group		Sig chi^2
	Pre	Post	Pre	Post	P* value
FBG	41	79	54	48	<0.05
PPBG	39	97	58	49	<0.05

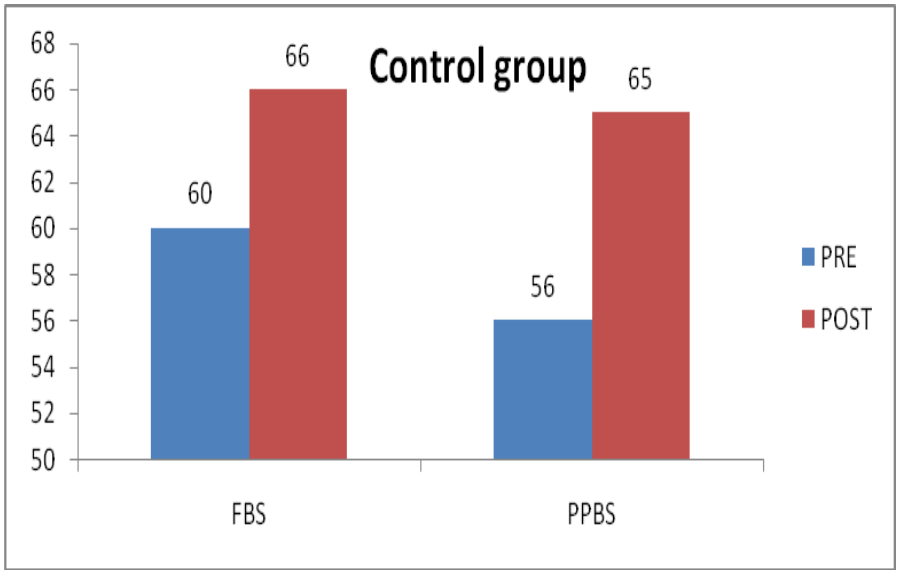
*chi square test. Significantly lesser number of pts with T2DM were in pathological range of glucose values in *Yoga* than control group after 3 months Chi-square test) higher in *Yoga* than the non- *Yoga* group.

Fig 14: Study 1- multicenter trial -Number of participants with pathologic values for FBG and PPBG before and after *Yoga* intervention



There is significant decrease in the number of participants with pathologic values of FBG and PPBG following *Yoga* intervention.

Fig 15: Study 1- multicenter trial -Number of participants with pathologic values for FBG and PPBG before and after 3 months in control group.



There is significant increase in number of participants with pathological values of FBG and PPBG in control group.

Study 2: RETROSPECTIVE STUDY

Title: Effect of Residential Integrated *Yoga* Program (RIYP) on Blood Glucose Levels, physiological variables and anti-Diabetes medication requirement in Type 2 Diabetes Patients: A Retrospective Study

4.6. Introduction

Lifestyle modification is the corner stone in primary and secondary prevention of T2DM. Studies have shown the benefit of *Yoga* -based life style modification in T2DM. It is well known that one should be regular about the four major factors of life style namely diet, physical activity, restraint from wrong eating, sleeping and addictive habits, and stressful responses to demanding situations of life.

The problems of habituated patterns of behavior are difficult to overcome. Hence intensive programs in residential set ups in which patients can experience healthy regular life style is advocated. There are many residential wellness centers in India designed for individuals with lifestyle diseases. VYASA *Arogyadhama* is one such center situated in south of Bengaluru, in a serene 100-acre campus that hosts facility for individuals to experience a daily routine of intensive lifestyle modification with a daily routine from 5-30 am till 10 pm; pre-post data in patients with diabetes are documented as a part of their program. The present retrospective study is a compilation of the results of these cases with T2DM from different parts of India and abroad who were admitted to *Arogyadhama* for two weeks between 2013 to 2015.

4.7. Aims and objectives

4.7.1. Aim

To understand the impact of 2 weeks of Integrated *Yoga* (IY) on fasting and post-prandial blood glucose level among patients with T2DM.

4.7.2. Objectives

1. To study the effect of 2 weeks of intensive *Yoga* -based lifestyle (IAYT) intervention in a residential set up on FBG and PPBG, in type 2 diabetes mellitus.

2. To study the effect of 2 weeks of intensive *Yoga* -based lifestyle (IAYT) intervention in a residential set up on glucose homeostasis, in type 2 diabetes mellitus.

4.8. Hypothesis

Two weeks of residential *Yoga* intervention reducesfasting and post-prandial blood glucose levels in Type 2 diabetes mellitus patients.

4.9. Null hypothesis

Two weeks of residential *Yoga* intervention cannot reduce fasting and post-prandial blood glucose levels in Type 2 diabetes mellitus patients.

4.10. Material and Methods

From a holistic health center Vivekananda *Yoga* Arogyadhama, in Bengaluru, India, data of 598 (186 females) T2DM patients admitted between January 2013 and December2015 were compiled retrospectively from their hospital medical records.

4.11. Inclusion and exclusion criteria

Participants' records were included on matching parameters criteria laid down for the selection. All cases with 1) T2DM and 2) On oral hypoglycemic medication were included. Records of patients with 1) previous history of long-term *Yoga* practice, 2) psychiatric diagnosis, 3) been on insulin therapy or 4) had been admitted for gestational diabetes or 5) type 1 diabetes were not considered.

All participants underwent a 15-day RIYP program which involved a time table based supervised *Yoga* -based lifestyle change with components of regulated sleep, *yogic* Sattvic diet, Asanas, pranayama, relaxation techniques, meditations, *Yoga* -based cleansing procedures, lectures on *Yoga* philosophy and selfless service.

They were assessed before and after intervention for changes in fasting blood glucose, post-prandial blood glucose, medication scores, symptom scores, systolic and diastolic blood pressure, bhramari time (mean of 3 exhalation time while making a low-pitched sound like a bee), pulse rate and respiratory rate.

4.12. Ethical Considerations

Study was approved by the Institution Ethical Committee of S-VYASA University, Bengaluru, India. All participants in the study had already signed informed consent form

as a part of their admission formality, in which they had accepted to permit the institution to use the details of their parameters documented in their files for any research purpose without revealing their identity.

4.13. Intervention

The RIYP which is based on Integrated Approach of *Yoga* Therapy (IAYT) can be understood as a holistic model, which corrects the imbalances at physical, mental, intellectual and emotional levels through application of multiple components such as kriyas (cleansing techniques), *yogic* diet (simple wholesome vegetarian diet with moderate spices and in moderate) , *āsana*, loosening exercises, breathing exercises, *prāṇāyāma*, cyclic medication, mind sound resonance technique, devotional sessions ,yogic counseling and lectures). Rightly as the author put it, "no component singularly can claim to be the IAYT, nor could possibly have the same effects as the whole model,".

Table 12 and 13 show the details of Time table followed during 15 days of RIYP

Table 12: Time Table Followed during Residential Integrated *Yoga* Program (RIYP)

Time	Activity	Time	Activity
05.00 AM	Ablution	03.00 PM	Cyclic meditation
05.30 AM	Prayer (Prathasmaran)	04.00 PM	Asana / Special yoga technique
06.00 AM	Asana / Special yoga technique	05.00 PM	Tuning to nature
07.15 AM	Friendship meet (Maitri milan)- Gita sloka chanting and discourse (Satsang)	06.00 PM	Devotional session (Bhajan)
08.00 AM	Breakfast	06.45 PM	MSRT (Mind sound resonance technique)
09.30 AM	Yogic Counselling	07.30 PM	Dinner
10.30 AM	Pranayama	08.30 PM	Happy assembly (yoga game session)/cultural program
11.30 AM	Milk or ayurvedic tea (Malt)	09.15 PM	Group discussion/self practice
12.05 PM	Special yoga techniques	10.00 PM	Lights off
01.00 PM	Lunch and rest		

Table 13: Study 2- RIYP- Details of Special *Yoga* Technique for T2DM			
	Name	**Posture**	**Practices**
1.	Breathing practices (5 minutes)	Standing	*Hands Stretch Breathing*
		Sitting	*Rabit breathing* *Tiger Stretch Breathing*
2.	Loosening practices *Śithilikaraṇa vyāyāma* (5 minutes)	Standing	*Pādahastāsana* *Ardhacakrāsana vyāyāma*
			Trikoṇāsana vyāyāma
			Kaṭi-parivrattāsana (Spinal Twist)
		Sitting	*Cakkicālana*
			Bhujaṅgāsana
		Supine	*Pavanamuktāsana kriyā*
		Prone	*Dhanurāsana* Swing
3.	Relaxation (5 minutes)		Instant Relaxation Technique
4.	*Sūrya namaskāra* (5 minutes)		12 steps
5.	Asanas (10 minutes each)	Standing	*Ardhakaṭicakrāsana*
			Parivrattatrikoṇāsana
		Sitting	*Vakrāsana*
			Ardhamatsyendrasana
		Prone	*Bhujaṅgāsana* *Dhanurāsana*
		Supine	*Pavanamuktāsana* *Matsyāsana*
	Relaxation (10 minutes)		Deep Relaxation Technique
6.	*Kriyā*		*Kapālabhāti* *Vamanadhauti* (Once a week)
7.	*Prāṇāyāma* (10 minutes)		*Nādīśuddhi*
			Bhramarī prāṇāyāma
			Uccāraṇa
8.	Meditation (20 minutes)		*Cyclic Meditation*

4.14. Assessments

4.14.1.Blood glucose

Fasting (after 8 hours fasting) and post-prandial (2 hours after breakfast) blood glucose was assessed using venous blood before and after 14 days. Blood test were carried out in an authentic NABL accredited laboratory.

Systolic and diastolic blood pressure was measured using sphygmomanometer after 5 minutes of rest, at the baseline and after 15 days.

Symptoms severity was assessed on 4-point scale score 0-3 (0 = no symptom and 3 – severely symptomatic).

4.15. Results

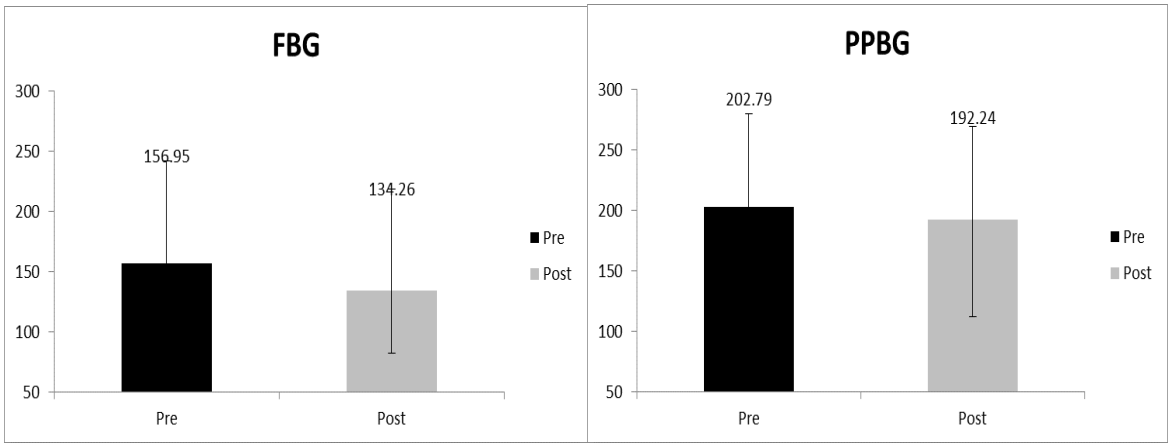
Analysis was done on data obtained from 598 files that had complete information necessary for this study. of these there were 186 females and 412 males in the age range of 56.45±11.02 years with a mean duration of DM of 8±3.4 years; T2DM was diagnosed, as per the ADA (American Diabetes Associaation) criteria.

The results of this study showed significant decrease in FBG (47%) and PPBG (28%) after two weeks of residential *Yoga* intervention compared to the baseline (depicted in table 3). Anti-diabetic medication score reduced significantly from 4.7 to 3.8.

Table 14: Showing pre-post changes in variables after 15 days of RIYP in T2DM patients.

Variables	**N**	**Pre (Mean ± SD)**	**Post (Mean ± SD)**	**mean diff**	**95% Confidence Interval of the Difference**		**P Value**
					Lower	**Upper**	
FBG (mg/dl)	**568**	156.95±84.82	134.26±51.46	22.69	-47.055	-35.645	<0.001**
PPBS (mg/dl)	**568**	202.79±77.29	192.24±79.62	10.55	-28.974	-16.394	<0.001**
Paired samples t-test							

Fig 16: Study 2 - RIYP-Pre-post changes in fasting and post-prandial blood glucose levels after 15 days *Yoga* intervention.



There was significant decrease in FBG and PPBG after 15 days of residential *Yoga* intervention

FBG and PPBG after *Yoga*: Significant decrease in fasting (from 156.95±84.82 to134.26±51.46; $p < 0.001$) as well as post-prandial blood glucose levels (from 202.79±77.29 to 192.24±79.62; $p<0.001$).

Table 14 above shows the number of participants with abnormal values before and after *Yoga*. There was significant reduction ($p<0.001$ chi^2) in those who presented with high values on admission.

Table 15: Number of participants in high values of FBG and PPBG before and after *Yoga*

Glucose level	N Before *Yoga*	N After *Yoga*	P Chi^2
FBG > 120mg/dl	204	136	<0.05
PPBG > 180mg/dl	264	113	<0.05

Chi^2 test showed that number of participants with pathologic score for FBG and PPBG significantly reduced after 15 days of IAYT intervention.

4.15.1. Changes in medication score

The number of oral hypoglycemic medication in one day was tabulated. The medication was reduced only when the blood glucose levels fell below acceptable levels. Table shows the changes in mean and SD of the number of tablets of oral hypoglycemic tablets which reduced significantly.

Table 16: Changes in medication score

Variables	Pre (Mean ± SD)	Post (Mean ± SD)	df	95% Confidence Interval of the Difference		P[a] Value
				Lower	Upper	
Medication Score	4.76±3.30	3.88±3.20	597	19.313	23.623	<0.001**
Oral hypoglycemic medication requirement reduced significantly (p<0.001) from average of 4.76/day to 3.88/day						

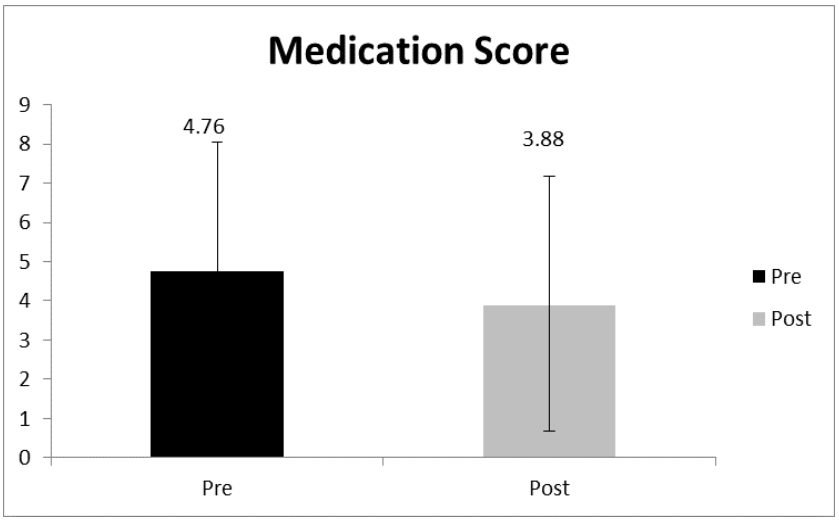


Fig 17 : Changes in Medication Score

Physiological variables:

Changes is cardiac variables

A significant decrease in pulse rate (from 79.63±9.24 to 60.93±27.79; p<0.001), systolic blood pressure (126.8 to 108 mmHg) and diastolic blood pressure (74.6 to 63.1 mmHg) was observed after 15 days of intervention compared to the baseline. Standard deviation also

reduced significantly for all three variables after 15 days compared to the baseline. This is suggestive of improvement in cardiovascular homeostasis.

Table 17: Study 2-RIYP-changes after 15 days of residential *Yoga* in T2DM patients

Variables	Pre (Mean ± SD)	Post (Mean ± SD)	Df	95% CI of df		Pª Value
				Lower	Upper	
Pulse Rate (bpm)	79.63±9.24	60.93±27.79	597	86.12	55.82	<0.001
Systolic BP (mmHg)	126.81±18.08	108.08±24.46	597	-37.37	-30.482	<0.001
Diastolic BP (mmHg)	74.6±10.45	63.13±31.53	597	15.37	20.365	<0.001

Figure – 18: Changes in pulse rate

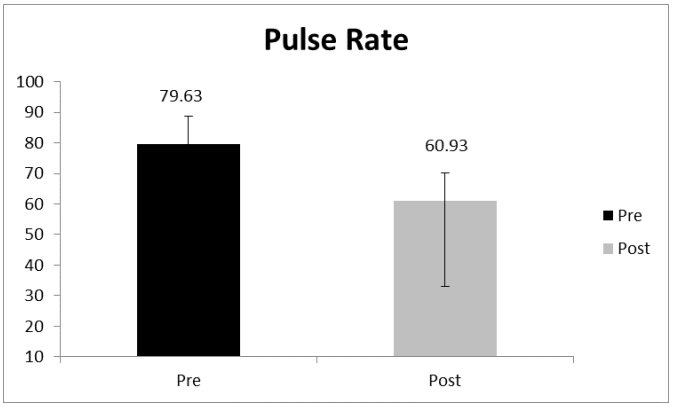
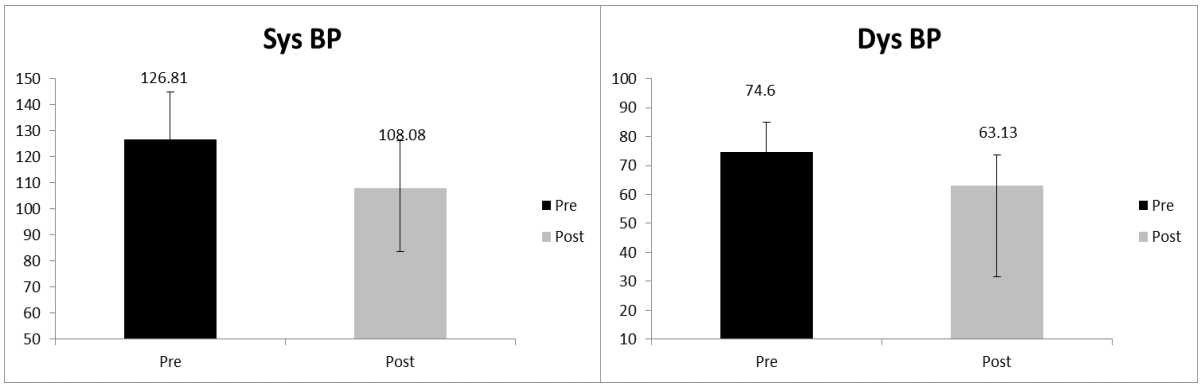


Figure - 19: Changes in blood pressure



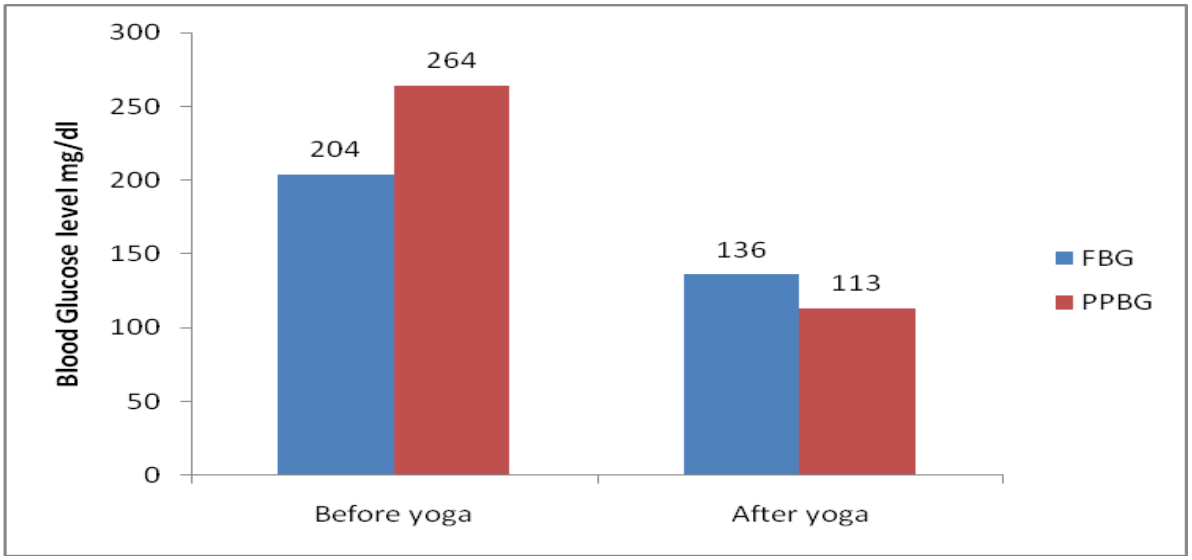
4.15.2. Changes in Respiratory rate

A significant decrease in the respiratory rate (from 18.28±3.7 to 15.66±4.38; $p<0.001$) was observed after 15 days of intervention compared to the baseline. Standard deviation also reduced significantly after 15 days compared to the baseline.

Table 18: Study 2-RIYP-changes after 15 days of residential *Yoga* in T2DM patients.

Variables	Pre (Mean ± SD)	Post (Mean ± SD)	df	95% CI of df		P[a] Value
				Lower	Upper	
Respiratory Rate (bpm)	18.28±3.71	15.66±4.38	597	16.84	20.556	<0.001

Study 3: Effect of long-term practice of integrated *Yoga* on insulin dynamics, systemic homeostasis, and gene ontology in patients with T2DM: A Cross-sectional study



Study 3: Effect of long-term practice of integrated *Yoga* on insulin dynamics, systemic homeostasis, and gene ontology in patients with T2DM: A Cross-sectional study

To bring about reversal of subtle level changes at genetic and molecular level, it may take long duration of healthy lifestyle. Hence the present study aims to understand the mechanism of systemic homeostasis in T2DM patients who are long term regular *Yoga* life style practitioners and comparing with non- *Yoga* practitioners managed by conventional method.

4.16. Aim

- To study the effect of *Yoga* on systemic homeostasis in T2DM in long term *Yoga* practitioners

4.17. Objectives

1. To compare the effect of long-term *Yoga* -based lifestyle (IAYT) intervention with that on conventional management for T2DM, on glucose homeostasis, in T2DM.
2. To compare the effect of long-term *Yoga* -based lifestyle (IAYT) intervention with conventional management of T2DM, on insulin dynamics.

3. To compare the lipid profile of T2DM patients who are long term *Yoga* lifestyle (IAYT) practitioners with those of conventional management
4. To study the effect of long-term *Yoga* -based lifestyle (IAYT) practice on renal functions
5. To study the effect of long-term *Yoga* -based lifestyle (IAYT) practice on stress measures
6. To study the effect of long-term *Yoga* -based lifestyle (IAYT) practice on gene expression
7. To study the effect of long-term *Yoga* -based lifestyle (IAYT) practice on metabolic and molecular homeostasis.

4.18. Hypothesis

Long term *Yoga* -based lifestyle program can bring about better functioning and homeostasis in metabolic and molecular variables in patients with T2DM

4.19. Null hypothesis

Long term *Yoga* -based lifestyle program may not bring about favorable changes in all metabolic and molecular variables in patients with T2DM

4.20. Design of the study

This was a two-armed cross-sectional study to assess the impact of long-term *Yoga* practice in type 2 diabetes.

4.21. Materials and methods

4.22. Source of participants

Study group of T2DM cases who were long term *Yoga* practitioners was selected after a thorough interview of their regularity of *Yoga* practice through interviews and also verifying their attendance in the centers for daily regular practice. Most of them were patients who had attended residential program at the residential health home and were continuing to practice at city center in Bengaluru, or in their residence or in other cities where regular daily *Yoga* classes are held. This cross-sectional study included a total 42 participants with T2DM of age 40 years and above. Of these, 20 participants were long term *Yoga* practitioners and 22 were non- *Yoga* practitioners.

4.23. Inclusion criteria

For *Yoga* group, participants with T2DM who were regular *Yoga* practitioners (minimum one hour, 3 days in a week) for at least 5 years in the immediate past were included. Control group included T2DM patients who were non- *Yoga* practitioners and were matched for age, gender and duration of diabetes.

4.24. Exclusion criteria:

Participants with history of T2DM less than 10 years, amputation of limbs, severe neuropsychiatric problems, taking insulin, were excluded. Those with associated major complications of diabetes such as renal failure, history of coronary or cerebra-vascular complications or proliferative retinopathy were excluded. Those with uncontrolled severe level of hypertension, severe obesity (BMI> 40) were also not included.

4.25. Ethical clearance and informed consent

Ethical clearance was obtained from Institutional Ethical committee of S-VYASA University, Bengaluru. All participants signed written informed consent form before the study.

4.26. Statistical analysis

All statistical analyses were done using SPSS version 20. Data were present as mean and standard deviation. Within group and between group differences were assessed using parametric and non-parametric test depending upon distribution of the data.

4.27. Results

4.27.1. Demographics

In this cross-sectional study there were a total of 42 participants with T2DM who satisfied the selection criteria. Of these, 20 were long term *Yoga* practitioners who had practiced *Yoga* regularly for at least 5 years in the immediate past (*Yoga* group) before recruitment and 22 participants were non- *Yoga* practitioners (Control group).

4.27.2. Family history of T2DM

Positive family history of T2DM has been recognized as one of the risk factors for T2DM. In the study total 26 participants (60%) (12 participants from the *Yoga* group and 14 participants from the control group) had a family history of T2DM.

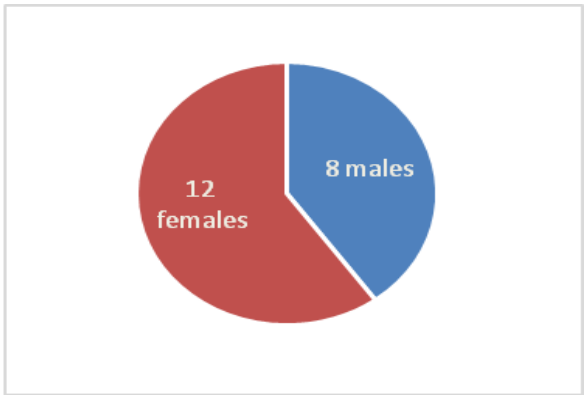
Table 19: Study 3 - Long term *Yoga* cross sectional-controlled study-Demographic characteristics of participants.			
Variables	***Yoga* (n=20)**	**Non-*Yoga* (n=22)**	**Sig P***
Gender distribution	Males=8, females=12	Males=11, females=11	NS[B]
Age (years)	63.7±9.06	61.15±7.47	NS[B]
Occupational History	**Females:** Homemakers-7 Services-3 **Males:** businessmen- 3 Service-2 Retired-4	**Female:** homemakers-9 Retired services -2 **Males:** services-2 retired-11	**NS**[B]
History of T2DM (years)	11.7±7.6	10.62±6.4	NS[B]
Family history of T2DM	13	15	NS[B]
Hypertension	8	13	NS[B]
Diabetes neuropathy	0	1	NS[B]
Diabetes retinopathy	0	0	NS[B]
Hypothyroidism	2	3	NS[B]
No co-morbidity	8	1	NS[B]
Dyslipidaemia	3	6	NS[B]
PSS	14.08±6.02	16.40±6.02	<0.001[A]
Diabetes Distress Scale	20±4.8	43.31±14.06	<0.001[A]
*[B] Chi-square test; [A-] Wilcoxon's Signed rank t test			

The *Yoga* group consisted of 20 participants with mean age 63.7±9.06 years; among these there were 8 Male and 12 female participants. In the control group 11were males and 11 were females with a mean age of 61.15±7.47 years.

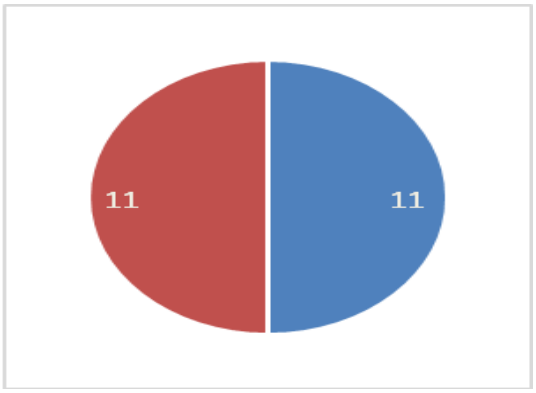
Table 20: Gender distribution across the groups

Variables	*Yoga* (n=20)	Non-*Yoga* (n=22)	Sig. P*
Gender distribution	Males=8, females=12	Males=11, females=11	NS[B]

Figure: 20 *Yoga* group N=20



Non- *Yoga* N=22

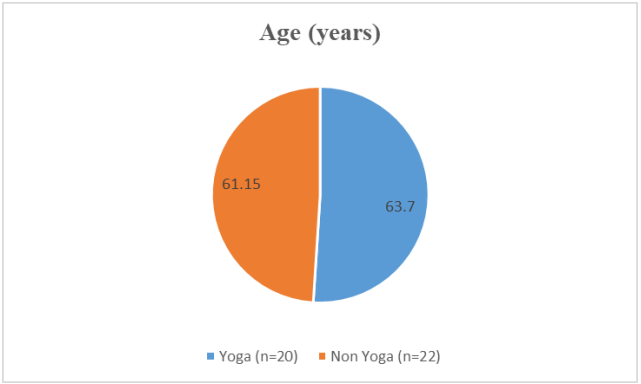


Mean age of the participants in the *Yoga* group was 63.7±9.06 years and for the control group 61.15±7.47 years. There was no significant difference between the groups in terms of age.

Table: 21

Average age of the participants in *Yoga* and non- *Yoga* group			
Variables	***Yoga* (n=20)**	**Non-*Yoga* (n=22)**	**Sig P***
Age (years)	63.7±9.06	61.15±7.47	NS[B]

Fig 21.: Mean age in *Yoga* and non-*Yoga* group



Participants in the *Yoga* group had practiced *Yoga* life style program for a minimum of 2 years of monitored regular practice. Most of the female participants from both the groups were homemakers and most of the male participants were retired persons. The average

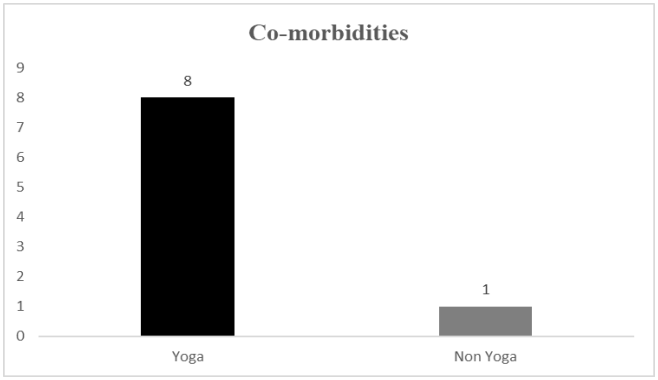
history of T2DM for the *Yoga* group and the control group was 11.7±7.6 and 10.62±6.4 years respectively (See table 19 above in previous pages).

Table: 22 - Occupational History				
Gender	**Occupation**	***Yoga* (n=20)**	**Non-*Yoga* group (n=22)**	**Sig**
Males	Service	2	2	**NS[B]**
	Retired	4	11	
	Businessmen	3	-	
Females	Homemakers	7	9	
	Service	3	2	

4.27.3. Co-morbidities

T2DM being a metabolic syndrome, it leads to several other metabolic conditions. Hypertension, stroke, heart disease, are frequently observed co-morbidities among patients with T2DM. In the studied population 8 (40%) participants reported no co-morbidity, whereas in the control group one (0.25%) participant was without any co-morbid condition. This indicates that patients with T2DM practicing *Yoga* have less chance of developing commodities compared to non- *Yoga* practitioners.

Figure 21: Participants with no co-morbid condition in *Yoga* and non-*Yoga* group

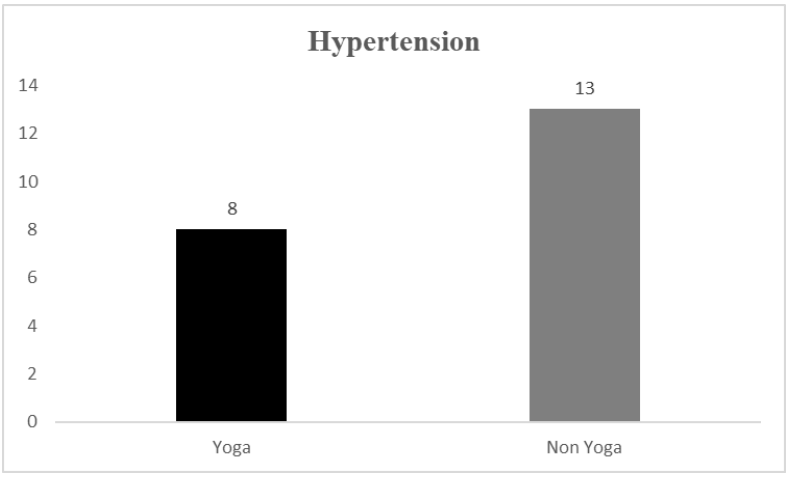


4.27.4. Hypertension

Hypertension has been the most common co-morbid condition observed in T2DM patients. Hypertension increases the risk of cardiac disease, renal complications and stroke in T2DM.

In the present study, of the total of 42 participants, 21 (47%) participants had hypertension:8 (40%) in *Yoga* group and 13 (60%) in the non- *Yoga* group. These findings indicate that *Yoga* practice is associated with decreased risk of hypertension among T2DM.

Figure 22: Number of participants having hypertension in *Yoga* (n-8) and non-*Yoga* (n-13) groups

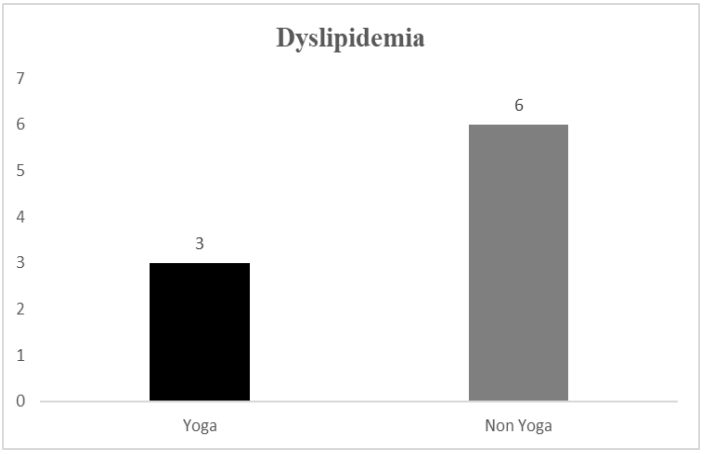


4.27.5. Dyslipidaemia

Six participants in the non- *Yoga* group and 3 from *Yoga* group had dyslipidemia.

Further, Diabetes retinopathy, Diabetes neuropathy are the most common complications of T2DM. In this study, none of the participants from either group had Diabetes retinopathy and one participant from the control group had Diabetes neuropathy.

Figure 23 Number of T2DM individuals with dyslipidemia in *Yoga* (3) and non-*Yoga* (6) groups



4.27.6. Comparison of Biochemical variables

Table 23: Study 3 – long term *Yoga* cross sectional-controlled study- Mean and SD of biochemical variables in *Yoga* and non- *Yoga* groups

Variables		***Yoga***	**Non-*Yoga***
Stress hormone	**Cortisol µg/dL**	12.8213.14	22.24±24.35
Cytokine	**IL-2**	36.36±11.29	33.18±8.87
Liver function test	**ALT (IU/L)**	13.74±5.78	17.75±9.19
	AST(IU/L)	21.22±4.63	27±12.3
	ALB(g/dL)	47.83±2.69	46.75±5.26
	D Bil-V(mg/dL)	0	0.04±0.2
	ALP(IU/L)	81.13±27.32	95.29±61.04
	Glu (mmol/L)	187.96±112.64	199.38±83.46
Renal function test	**Urea**	29.78±9.48	33.58±15.71
	Cre(mg/dL)	1.01±0.28	1.15±0.76
	Urea(mg/dL	5.43±1.53	5.92±1.86
Psychological questionnaires	**DSS**	20±4.81	43.32±14.06
	PSS	14.79±6.03	16.41±6.03

4.27.7. C-Peptide

Connecting peptide (C-peptide) is hormone produced in the beta cell of the pancreas. C-peptide is produced in equimolar amounts to insulin. Hence, the C-peptide measure is used as an alternative to insulin measurement. In the present study, we found that mean value for C-peptide was lower in the *Yoga* group (36.54±20.81) compared to the non- *Yoga* group (55.12±43.40). This could be due the fact that, T2DM is characterized by insulin resistance, which stimulates the excess production of insulin by beta cells leading to hyperinsulinemia. Decrease in the C-peptide in the *Yoga* group could be due to decreased insulin resistance which might have led to decreased C-peptide which corresponds to lesser need for insulin production.

Table 24 : Study 3 – long term *Yoga* cross sectional-controlled study- The Average C-Peptide level in the *Yoga* group and non- *Yoga* group.

Variable	***Yoga* group**	**Non- *Yoga* group**
C-Peptide	36.54±20.81	55.12±43.40

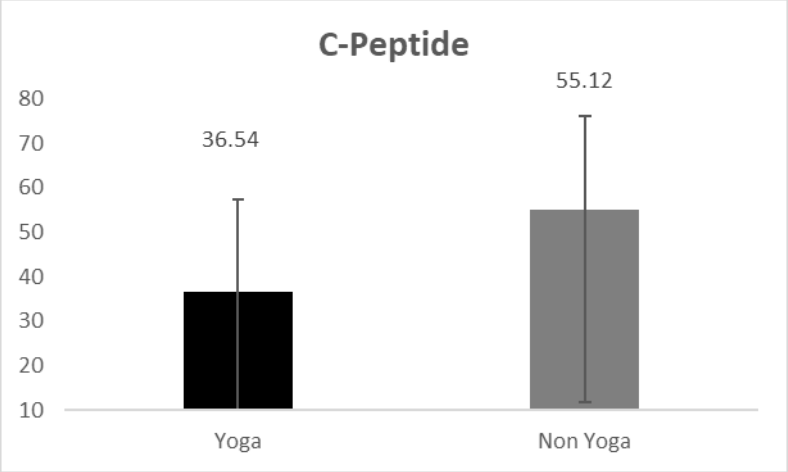


Fig 24*: Yoga* (36.54±20.81); Non- *Yoga* (55.12±43.40)

4.27.8. Pancreatic polypeptide

Pancreatic polypeptide (PP) is a polypeptide secreted by PP cells in the endocrine *pancreas* predominantly in the head of the *pancreas*. The function of PP is to self-regulate the secretion activities of both the endocrine and exocrine pancreas. PP is a regulator of pancreatic and gastrointestinal functions and has an important protein regulating food intake; it is a satiety hormone. Its levels are reduced in conditions associated with increased food intake and elevated in anorexia nervosa. Increased polypeptide indicates reduced requirement for insulin production.

Table – 25: Study 3 – long term *Yoga* cross sectional-controlled study		
PPT	***Yoga* (n=20)**	**Non- *Yoga* (n=22)**
<129pg/ml	16	13
pathologic values (>129pg/ml)	4	9

Fig - 25: Number of participants with normal value and pathologic value for pancreatic polypeptide in the *Yoga* and the non- *Yoga* group

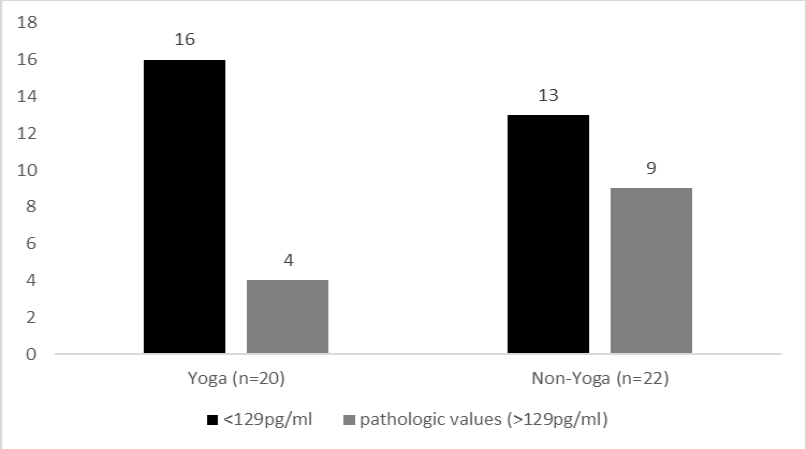


Fig 25*: Yoga* (n=20); Non- *Yoga* (n=22)

Pro-insulin to insulin ratio is considered to indicate the availability of active insulin. In the present study it is noticed that pro-insulin: insulin ratio in the *Yoga* group was lower (0.007) compared to the control group (0.11). This is suggestive of better insulin activity in the *Yoga* group compared to the non- *Yoga* group.

Table – 26: Pro-insulin to insul ratio in the ***Yoga*** group and the control group

Variable	*Yoga* **Mean (SD)**	**Non-** *Yoga* **Mean (SD)**	**P value**
Proinsulin: Insulin	0.007	11	<0.05

Liver profile

T2DM affects liver function also leading to increase in liver enzymes and fatty liver. In this study, it is found better liver function in the *Yoga* group compared to the control group. Aspartate amino transferase (AST) and Alanine aminotransferase (ALT) levels in the *Yoga* group were lower compared to the non- *Yoga* group. This is suggestive of better liver function is associated with the long-term *Yoga* practice.

Table – 27: Liver profile in *Yoga* and non- *Yoga* groups

Variable	*Yoga*		Non-*Yoga*	
	Mean	SD	Mean	SD
ALT (IU/L)	13.74	5.78	17.75	9.19
AST(IU/L)	21.22	4.63	27.00	12.30
ALB (g/dL)	47.83	2.69	46.75	5.26
D Bil-V (mg/dL)	0.00	0.00	0.04	0.20
ALP (IU/L)	81.13	27.32	95.29	61.04
Glutamine (mmol/L)	187.96	112.64	199.38	83.46
ALT - Alanine aminotransferase, AST – Aspartate amino transferase, ALB – Albumin, D Bil – Dir Bilirubin, ALP – Alkaline Phosphate, Glu – Glutamine milli-molar/L				

Figure :26 Liver profile parameters in the *Yoga* group and non- *Yoga* group of long- term *Yoga* effect.

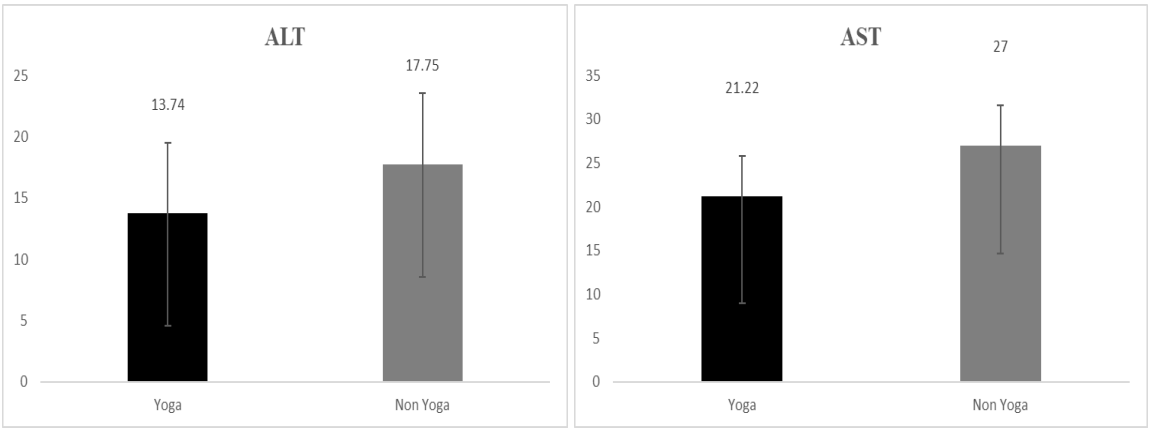


Figure: 27 ALB parameters in the *Yoga* group and non- *Yoga* group of long term *Yoga* effect.

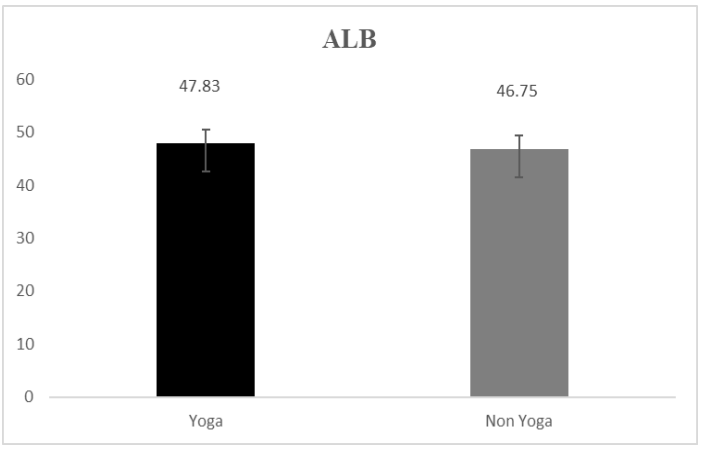


Figure: 28 ALP IU parameters in the *Yoga* group and non- *Yoga* group of long-term *Yoga* effect.

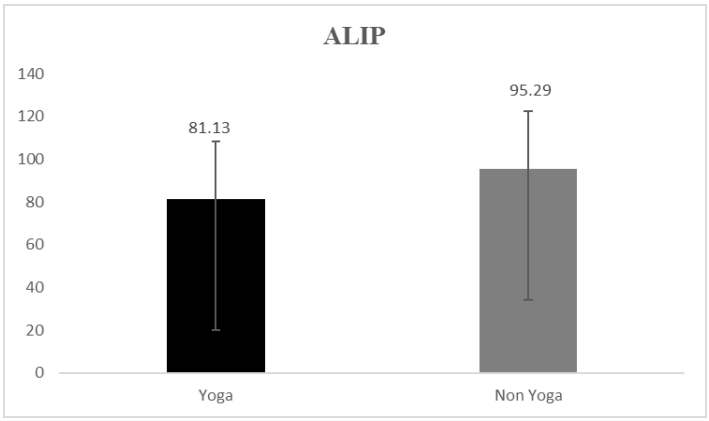
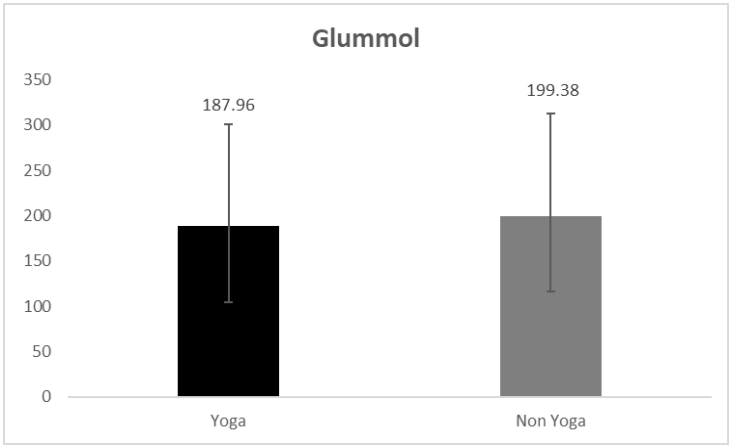


Figure: 29 Glutamine parameters in the *Yoga* group and non- *Yoga* group of long-term *Yoga* effect.



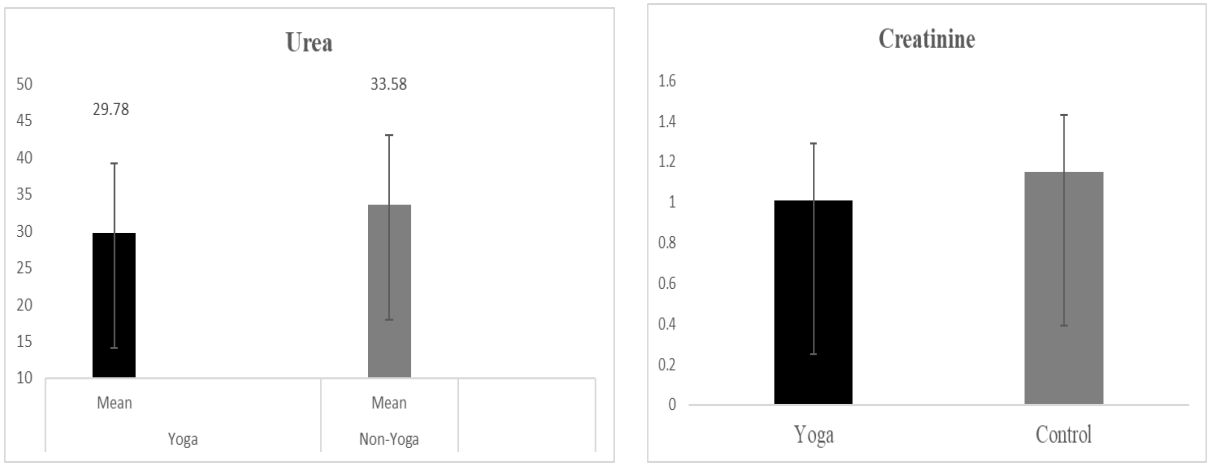
4.27.9. Renal Profile

Assessment of renal profile revealed an average creatinine level in the *Yoga* group was less compared to the control group; this is suggestive of the better renal function in among long term *Yoga* practitioners compared to the non- *Yoga* practitioners.

Table -28: Serum creatinine value in the *Yoga* and control groups

Variable	***Yoga***		**Control**	
	Mean	SD	Mean	SD
Urea	29.78	9.48	33.58	15.71
Creatinine mg/**dL**	1.01	0.28	1.15	0.76

Figure - 30: Blood urea & Creatinine levels in *Yoga* and control groups

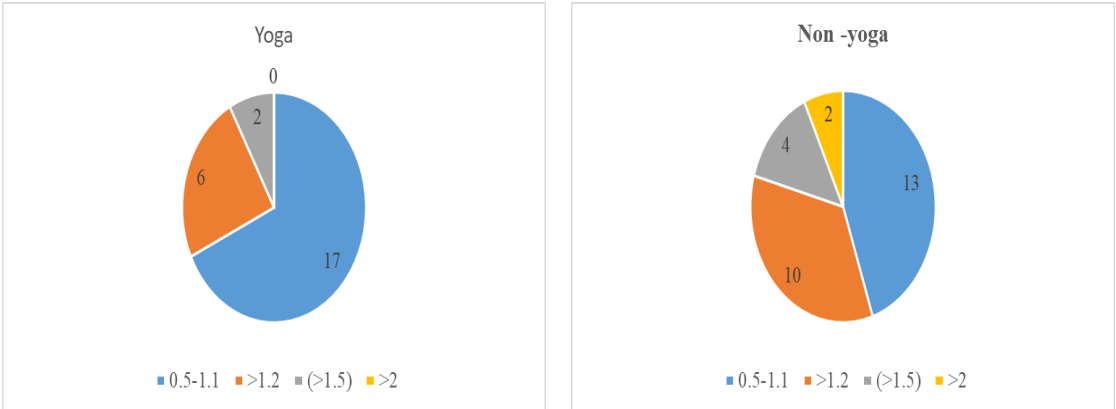


Further, it is observed that number of participants (17) with normal creatinine level in the *Yoga* group was more compared to the non- *Yoga* group (13). Four participants in the control group had pathologic value (>1.5), whereas, only two participants in the control group had pathologic value of creatinine. This is suggestive of *Yoga* practices may associate with decreased incidences of renal complication.

Table 29: Study 3 – long-term *Yoga* cross sectional-controlled study-renal profile

Number of participants in different ranges of creatinine values in the 2 groups		
Creatinine	***Yoga* (n=23)**	**Non - *Yoga* (n=23)**
0.5-1.1	17	13
>1.2	6	10
Number of Participants with pathologic values (>1.5)	2	4
>2	0	2

Figure: 31 Number of patients with different levels of creatinine values



More number of participants had creatinine value within the normal range in *Yoga* than control group.

Mean blood urea level in the *Yoga* group less compared to the control group

The study indicated that the mean values for kidney profile parameters (serum creatinine- 1.01±0.28, and blood urea – 29.79±9.48) in the *Yoga* group was lower compared to the control group (Serum creatinine - 1.15±0.76, and blood urea 33.58±15.71 Also, 4 participants from the control group and 2 participants from the *Yoga* group had a pathologic value for serum creatinine. These results suggest that T2DM participants practicing *Yoga* have better kidney function compared to non- *Yoga* practitioners. Further, these results also suggest the *Yoga* as a potential intervention to prevent kidney complication due to T2DM.

Table 30: Uric acid value for *Yoga* and non- *Yoga* group

Variable	*Yoga*	**Non-** *Yoga*
	Mean±SD	Mean±SD
Uric acid	5.43±1.53	5.92±1.86

Study 3 – Long term *Yoga*, cross sectional-controlled study. The mean serum uric acid level in the *Yoga* group and the non- *Yoga* group

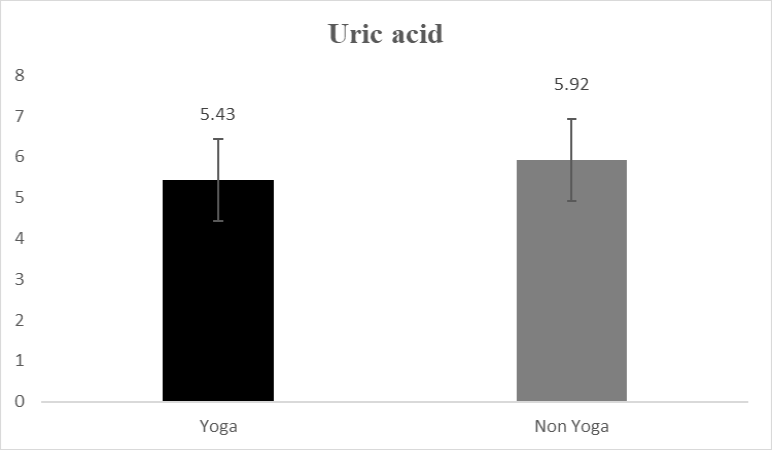


Figure 32: The mean serum uric acid in the *Yoga* group is lower (365.43) compared to the control group (5.92). Blood uric acid level (5.92±1.53) was lower in *Yoga* than non- *Yoga* group (5.92±1.86)

Study 3 – long term *Yoga* cross sectional-controlled study - mean urea level in the *Yoga* group and the control group

The mean urea in the *Yoga* group is lower (29.79) compared to the non- *Yoga* group (33.58)

Inflammation marker

Chronic systemic inflammation is common condition involved in pathology of T2DM. Systemic inflammation has been considered as one of the important contributing factors in development of insulin resistance. From the study it is assessed that the inflammation markers in long term *Yoga* practitioners (Hu, Meigs, Li, Rifai, and Manson, 2004). Il-2 levels were significantly higher in *Yoga* group (36.36±11.29) than non- *Yoga* group (33.18±8.87). IL-2 plays a major role in functioning of the immune system, tolerance and immunity, primarily via its direct effects on T cells. In the thymus, where T cells mature, it prevents autoimmune diseases by promoting the differentiation of certain immature T cells into regulatory T cells, which suppress other T cells which otherwise attack normal healthy cells in the body (Arenas-Ramirez, Woytschak, and Boyman, 2015). Regulatory T cells (T reg cells) play a major role in controlling the pathogenic autoimmune process in type 1 diabetes (T1D). Interleukin 2 (IL-2), a cytokine which promotes T reg cell survival and function, may thus have therapeutic efficacy in T1DM (Grinberg-Bleyer et al., 2010)

Table: 31 IL-2 in long term *Yoga* practitioners and non-practitioners

Variable	*Yoga* (Mean±SD)	Non-*Yoga* (Mean±SD)
IL-2	36.36±11.29	33.18±8.87

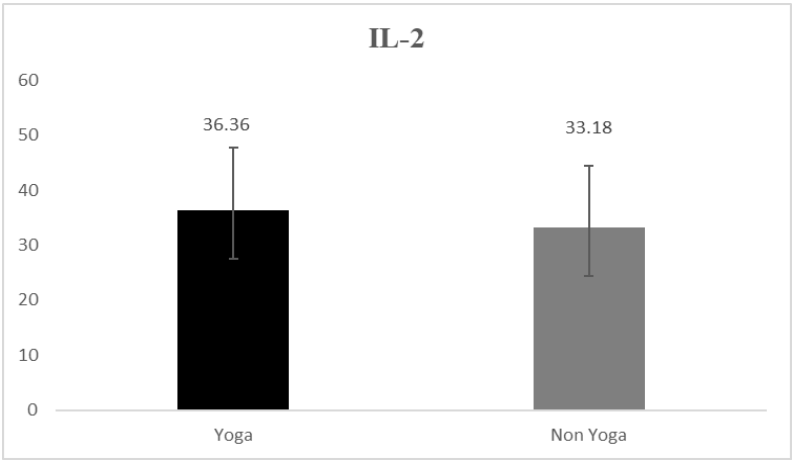


Figure 13: IL-2 level in the *Yoga* group is higher compare to non- *Yoga* group.

4.27.10. Stress profile

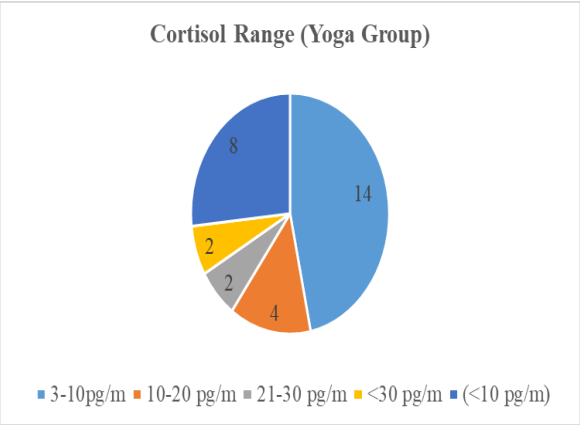
4.27.10.1. Serum Cortisol

High Serum Cortisol as a marker of stress levels is one of the cardiac risk factors. Increased serum cortisol levels in T2DM are associated with increased risk of acute myocardial infarction and hypertension among T2DM. In the present study, the mean values for serum cortisol was lower in the *Yoga* group (12.82±13.14) compared to the non- *Yoga* group (22.24±24.35).

Fourteen (63%) participants in the *Yoga* group and 11 (45%) participants in the non- *Yoga* group had normal serum cortisol (3-10 IU). It is noticed that 8 (36%) participants in the *Yoga* group and 13 (54%) participants in the control group had pathologic values for serum cortisol. This clearly establishes that participants with T2DM practicing *Yoga* have low cortisol levels compared to non- *Yoga* practitioners which could be due to decreased stress level which is also supported by decreased PSS values in the *Yoga* group. Decreased serum cortisol is also associated with decreased risk of cardiac complications and hypertension in T2DM.

Table 32: Study 3 – long term *Yoga* cross sectional-controlled study–
Number of participants in different ranges of serum Cortisol levels in the two groups

Cortisol range	***Yoga* (n=20)**	**Non-*Yoga* (n=22)**
3-10pg/m	14	11
10-20 pg/m	4	3
21-30 pg/m	2	2
<30 pg/m	2	8
Participants with pathologic values (<10 pg/m)	8	13



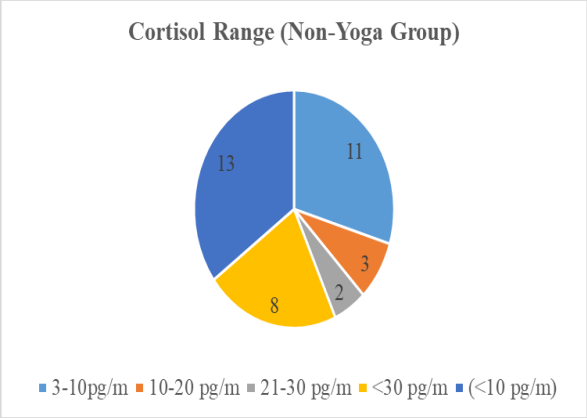
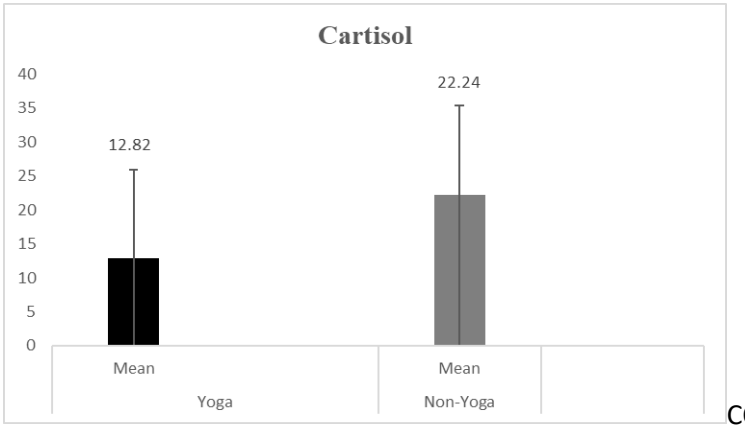


Figure 34: Mean and SD of serum cortisol in *Yoga* and Non-*Yoga* group



The mean Serum cortisol in the *Yoga* group is lower (12.82) compared to the non-*Yoga* group (22.24)

4.27.11. Lipid profile

Dyslipidemia is co-morbidity in T2DM which is associated with increased cardiovascular disease risk. In the present study the lipid profile was assessed with the long-term *Yoga* practitioners and control. We found that values for triglycerides, total cholesterol, low density lipid profile, very low-density lipoprotein in the *Yoga* group compared to the control group. This is suggestive of ant-dyslipidemia property of *Yoga*.

Table: 33 Lipid Variables in *Yoga* & Non- *Yoga* groups

Lipid variables	*Yoga*	**Non-** *Yoga*
	Mean	**Mean**
TC	176.78±44.84	178.83±46.7
TG	182.22±72.77	225.63±136.31
LDL	87.00±38.97	86.00±35.40
HDL	53.35±12.91	47.75±10.32
VLDL	36.43±14.53	45.08±27.28

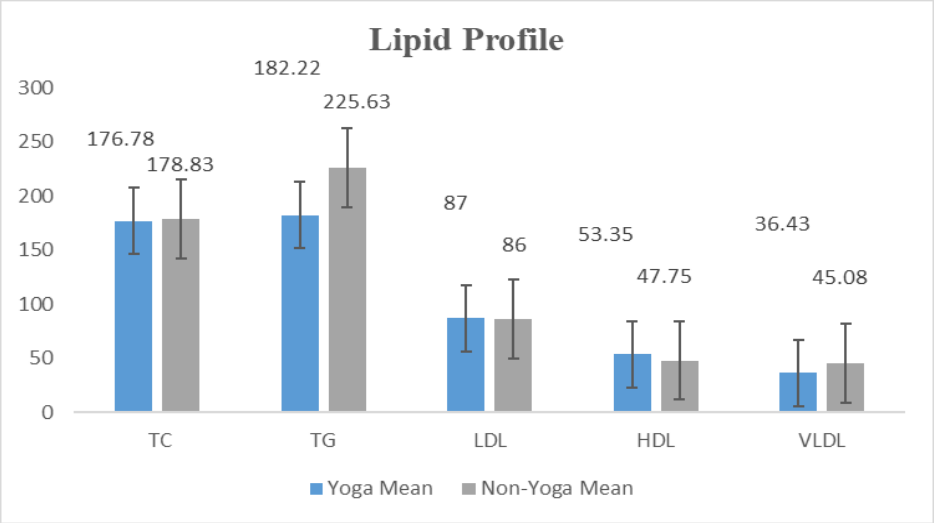


Fig – 35: Lipid profile mean values in *Yoga* & non- *Yoga* groups

4.27.12. Psychological variables

Assessed the subjective feeling of disease burden and also the efforts to manage T2DM by the patients, using diabetes distress scale.

4.27.12.1. Diabetes Distress Scale (DDS)

Diabetes Distress Scale (DDS) is a self-administered questionnaire with 17 items were administered to all the participants from both groups and found a low mean values for DDS in the *Yoga* group compared to the Non- *Yoga* group.

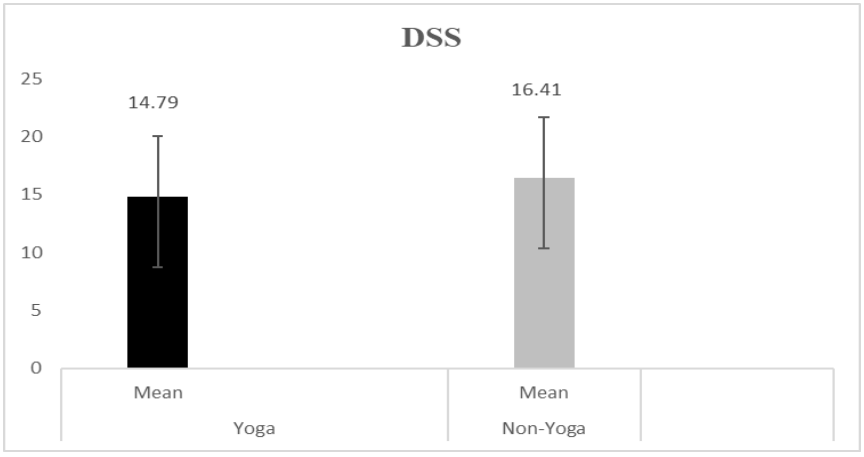
Diabetes distress is a measure of hidden emotional burdens and worries experienced by the patients during management of T2DM. The prevalence of Diabetes distress varies from 18-48%. High level of Diabetes distress is associated with poor glycemic control, low self-care and poor quality of life. DDS assessment relates to Diabetes management, depression and quality of life (QoL). A higher value indicates lower depression, higher efficacy of T2DM management and better QoL. It was found to be valid with Cronbach's alpha value of <0.70.

4.27.12.2. Diabetes Distress Scale (DDS)

Table 34: Study 3 – long term *Yoga* cross sectional-controlled Study-Diabetes distress scale

Variable	*Yoga*		Non- *Yoga*	
	Mean	SD	Mean	SD
DDS	20.00	4.81	43.32	14.06

Figure - 36: Study 3 – long term *Yoga* cross sectional-controlled study



The mean values for Diabetes distress scale values in the *Yoga* group lower (20) compared to the Non- *Yoga* group (43.32).

4.27.12.3. Perceived Stress

Perceived stress was assessed using Cohen's perceived stress scale (PSS). It is a valid and reliable tool to measure perceived stress. It is the most widely used psychological scale to measure the perception of stress. PSS consists of 10 items with 4-point Likert's scale ranging from 0-3. A higher score suggests a greater degree of perceived stress. PSS scores are obtained by reversing responses (e.g.0 = 4, 1 = 3, 2 = 2, 3 = 1, and 4 = 0) to the 4 positively stated items (items 4, 5, 7, and 8) and then summing across all scale items. A short 4-item scale can be made from questions 2, 4, 5, and 10 of the PSS 10-item scale (Cohen, 1994). It was found to be a valid tool with Cronbach's alpha values (0.82 for the full scale).

Table 35: Study 3 – long term *Yoga* cross sectional-controlled study- Perceived stress scale

Variable	*Yoga*		Non- *Yoga*	
	Mean	sd	Mean	Sd
PSS	14.79	5.24	16.41	6.03

Study 3 – long term *Yoga* cross sectional-controlled study-perceived stress values in the *Yoga* group and the Non-*Yoga* group

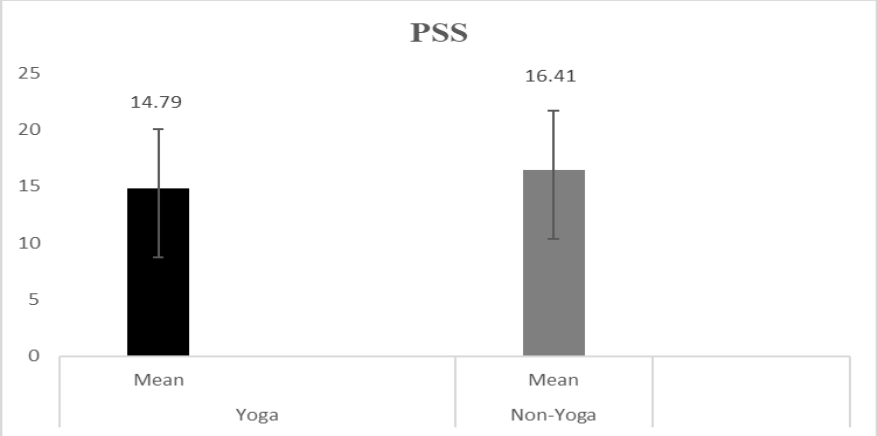


Fig 37: The mean PSS values in the *Yoga* group was lower (14.79±6.03) compared to the non- *Yoga* group (16.41±6.03).

4.27.13. Systemic metabolic Homeostasis

Type 2 Diabetes Mellitus is characterized by a disruption in glucose homeostasis (Özcan et al 2006) and it further, leads to dysfunction in various other organ functions leading to multi-organ dysfunction and disruption in systemic homeostasis. In our study, we assessed the functions of various organs such as kidney, liver and pancreas along with lipid profile parameters and cortisol levels in type 2 DM patients.

The systemic homeostasis is defined by considering two phenomena observed in our study.

a. Values of the variables within normal healthy range. We tabulated all variables to check this phenomenon of normalcy between the two groups.

b. Small SD with the mean value within the normal range of the given normative values.

It is noticed that 91% of the participants in the *Yoga* group and 46% of the participants in the Non- *Yoga* group had all the variables, including renal profile, liver profile, lipid profile, insulin profile, glucose profile and stress measures (cortisol) within the range of the normative values provided by the lab standards. This is suggestive of better homeostasis in the *Yoga* group as compared to poor homeostasis in the Non- *Yoga* group.

Table - 36: Study 3 – long term *Yoga* cross sectional-controlled study

Number of participants with systemic metabolic homeostasis in the *Yoga* was higher in *Yoga* than non- *Yoga* group.

Number of participants with systemic metabolic homeostasis in the *Yoga* and the Non-*Yoga* group						
Status	Non- *Yoga*		*Yoga*		Total	p chi^2
	n	%	n	%		
Homeostasis	13	59.09	19	95	32	NA
Non-homeostasis	09	40.91	1	5	10	NA

Figure: 38 Number of participants with systemic homeostasis is more in the *Yoga* group compared to the Non-*Yoga* group.

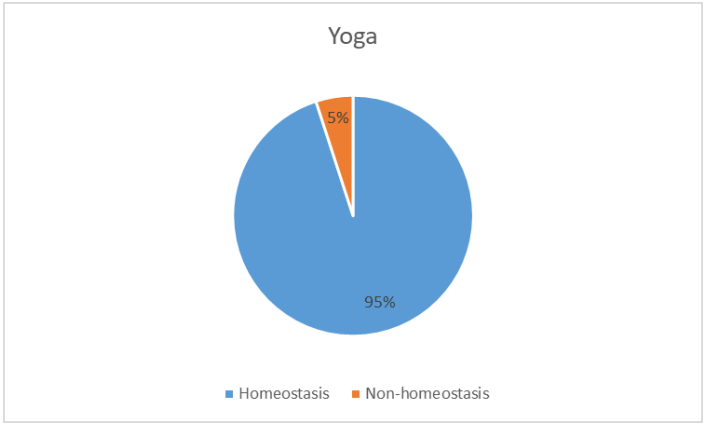
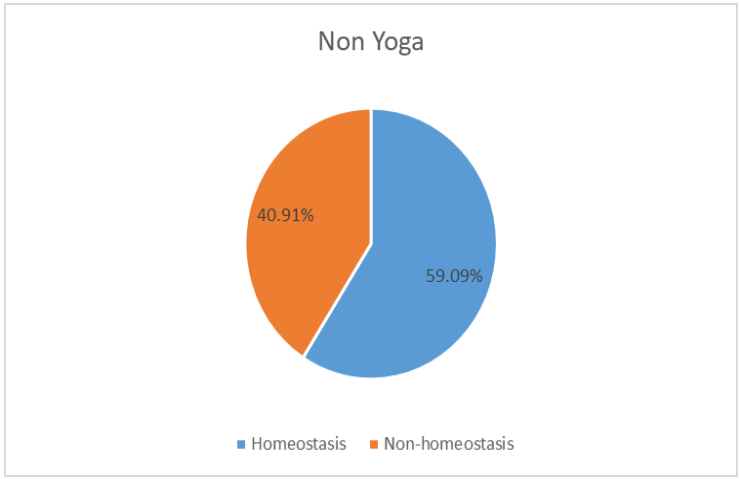


Figure: 39 Number of participants with systemic homeostasis is more in the *Yoga* group compared to the Non-*Yoga* group.



These results indicated that the long-term *Yoga* practice helps in maintaining better homeostasis in T2DM.

Table: 37 number of variables within the normal range

No. Tests	Non-*Yoga* group	*Yoga* group
9	7	6
10	4	6
11	1	4
12	5	7
13	1	0
Total	**18**	**23**

Study 3 – long term *Yoga* cross sectional-controlled study

Figure 40 Number of participants having optimal homeostasis in the *Yoga* & the Non-*Yoga* group

91% were in homeostatic range in *Yoga* group while only 54% of the participants in the Non-*Yoga* group had optimal homeostasis.

Standard deviation for 9 variables was 2 times higher in the Non- *Yoga* group compared to the *Yoga* group. Narrow standard deviation indicates less variation in the parameters from the mean, suggesting better homeostatic state.

Counted the number of tests within the normative range for each of the participants and tabulated them.

Study 3 - Molecular genetics assessments

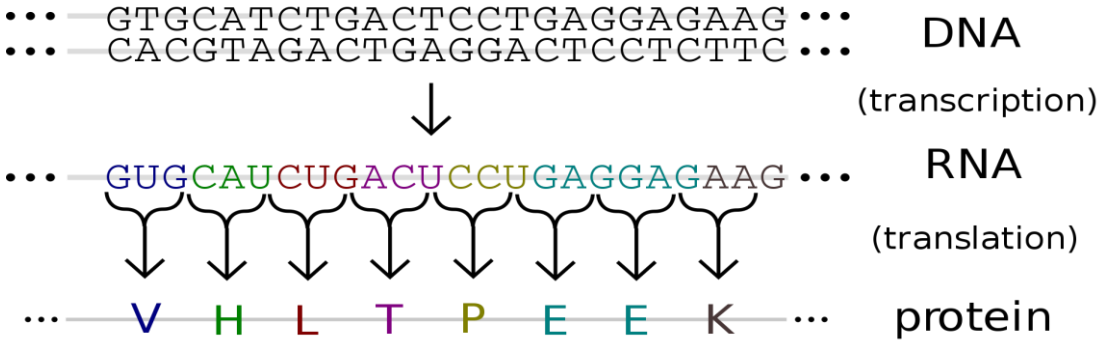
A gene is a stretch of DNA that encodes information. Genomic DNA consists of two anti-parallel and reverse complementary strands, the "template strand" which serves as a blueprint for the production of an RNA transcript, and the "coding strand' which includes the DNA version of the transcript sequence. The "coding strand" is not physically involved in the coding process because it is the "template strand" that is read during transcription.

The production of the RNA copy of the DNA is called transcription, and is performed in the nucleus by RNA polymerase, which adds one RNA nucleotide at a time to a growing RNA strand as per the complementarily law of the bases; the resulting RNA strand is identical to the coding DNA strand with the exception that thymine's are replaced with uracils (U) in the RNA.

4.27.13.1. Gene expression

In genetics, gene expression is the fundamental level of expression of the genotype to the phenotype, i.e. the observable trait. The genetic code stored in DNA is "interpreted" by gene expression that gives rise synthesis of proteins that non- *Yoga* the organism's shape, or that act as enzymes that promote specific metabolic pathways that decide the specificity of the organism.

Regulation of gene expression is thus critical to an organism's development.



Genes are expressed by being transcribed into RNA, and this transcript may then be translated into protein.

https://commons.wikimedia.org/wiki/File:Simple_transcription_elongation1.svg

4.27.13.2. Gene regulation

The complex gene expression which is characteristic of human genes, begins with non-*Yoga* of access to the DNA. This form of regulation, called epigenetic regulation, occurs even

before transcription is initiated. Gene regulation is the process of controlling which genes in a cell's DNA are expressed (used to make a functional product such as a protein). In eukaryotes like humans and gene regulation involves many steps. The expression of genes is controlled primarily at the level of initiation of transcription, although in some cases transcription may be attenuated and regulated at subsequent steps.

Regulation of gene expression depends on the accessibility of large regions of DNA which can depend on its chromatin structure that can be altered as a result of histone modifications directed by DNA methylation, ncRNA, or DNA-binding protein. Hence these modifications may up or down regulate the expression of a gene. Some of these modifications that regulate gene expression are inheritable and are referred to as epigenetic regulation.

4.27.13.3. Differential regulation of genes

A gene is declared differentially expressed if a difference or change observed in read counts or expression levels/index between two experimental conditions is statistically significant. Through the process of differential gene expression, the activation of different genes within a cell that define its purpose, each cell expresses only those genes which it needs. However, the extra genes are not destroyed, but continue to be stored within the nucleus of the cell.

4.27.13.4. Gene over-expression

Gene over-expression is the process which leads to the abundant target protein expression subsequently. The process may be in the cell where the gene is originally located or in other expression systems.

4.27.13.5. Lab Procedure for gene expression

Northern blot or serial analysis of gene expression (SAGE) are the techniques that make it possible to identify which genes are turned on and which are turned off within the cells. Subsequently, this information can be used to help determine what circumstances trigger expression of various genes.

Both Northern blots and SAGE analyses work by measuring levels of mRNA, the intermediary between DNA and protein. In order to activate a gene, a cell first copies the DNA sequence of that gene into a piece of mRNA known as a transcript. By determining which mRNA transcripts are present in a cell, scientists can determine which genes are

expressed in that cell at different stages of development and under different environmental conditions.

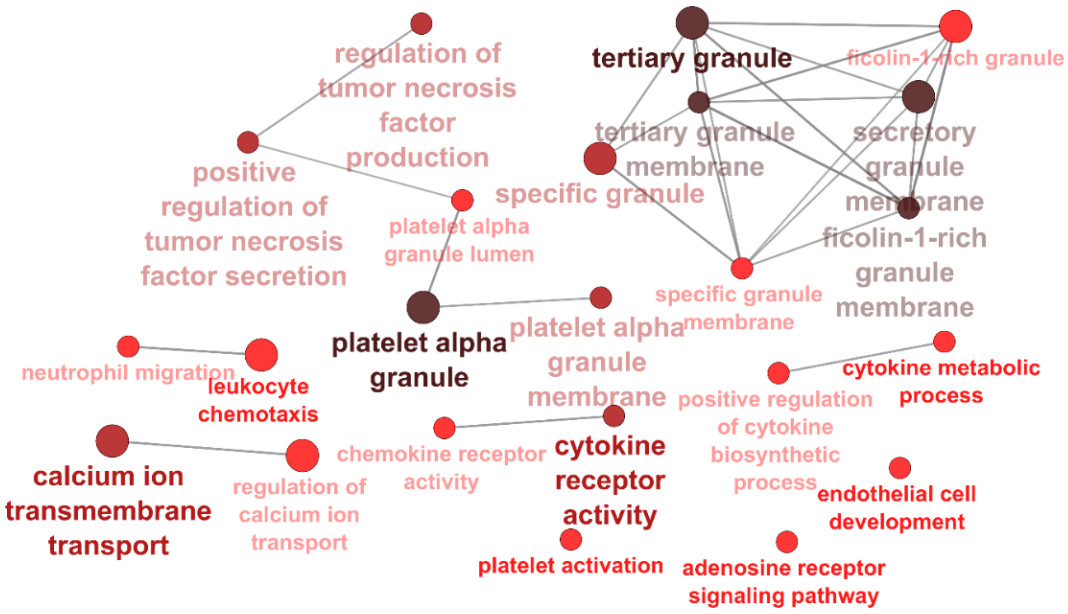
It is determined that the differentially expressed genes in this cross-sectional study on two groups of diabetics of study 3. Classification of Differentially Regulated Genes in T2DM: long term *Yoga* practitioners compared to non- Yoga practitioners.

Procedure: The Differentially Expressed Genes (DEG) was analyzed and the genes with more than two-fold differential expression between *Yoga* and non- *Yoga* groups were tabulated.

The DEGs of each condition were grouped according to the PANTHER protein class, GO Molecular Function, GO Biological Process and GO Cellular Component. Grouping the DEGs in every time point according to the protein class, biological process and molecular function were most useful. A total of 267 DEGs had annotations in the DAVID database. Fischer exact test was performed and the significant functional groups were classified further. Of these 99 functions classified under three groups namely the Biological Process, Molecular Function and Cellular Component were tabulated.

Biological Processes that were differentially expressed in *Yoga* as compared to non- *Yoga* group are tabulated (table 44)

Figure- 41 depicts the predominantly regulated pathways as seen using clue Gocytoscope



Gene Ontology results suggest that *Yoga* might regulate the effects in T2DM patients by regulating inflammatory process, platelet degranulation, immune system activity and cell signalling to decrease insulin resistance. Over representation analysis using ClueGo suggest that platelet regulation as one of the key processes regulated in the *Yoga* practitioners.

4.27.14. Significantly Differentially Regulated Pathways:

Significantly regulated pathways were tabulated from Consensus pathway database and tabulated. The results indicate that pathways associated with prevention of platelet aggregation were significantly regulated.

Table 38 Molecular markers

Pathway	**P-value**	**Q-value**	**Source**
Platelet activation, signaling and aggregation	3.45E-06	0.00154	Reactome
Immune System	5.90E-06	0.00154	Reactome
Cytokine-cytokine receptor interaction - Homo sapiens (human)	7.70E-06	0.00154	KEGG
Hematopoietic cell lineage - Homo sapiens (human)	3.10E-05	0.004643	KEGG
Platelet degranulation	5.19E-05	0.006228	Reactome
Response to elevated platelet cytosolic Ca2+	6.92E-05	0.006923	Reactome
Primary immunodeficiency - Homo sapiens (human)	8.84E-05	0.007576	KEGG
Eptifibatide Action Pathway	0.000163	0.009772	SMPDB
Tirofiban Action Pathway	0.000163	0.009772	SMPDB
Abciximab Action Pathway	0.000163	0.009772	SMPDB
Neutrophil degranulation	0.000189	0.009996	Reactome
Hemostasis	0.0002	0.009996	Reactome
Chemokine receptors bind chemokines	0.00043	0.019849	Reactome
Innate Immune System	0.00096	0.038692	Reactome
Platelet activation - Homo sapiens (human)	0.000967	0.038692	KEGG
Human Complement System	0.001155	0.043296	Wikipathways
Arrhythmogenic right ventricular cardiomyopathy (ARVC) - Homo sapiens (human)	0.002267	0.076705	KEGG

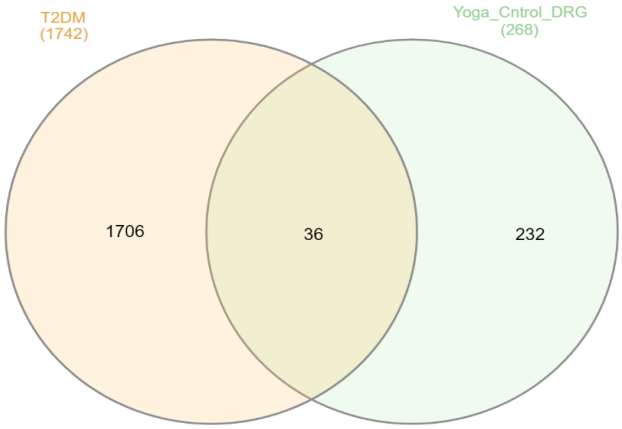
GPCR ligand binding	0.002305	0.076705	Reactome
Th17 cell differentiation - Homo sapiens (human)	0.002502	0.076705	KEGG
Arrhythmogenic Right Ventricular Cardiomyopathy	0.002557	0.076705	Wikipathways

In the above **table 38,** top 20 significantly regulated pathways

4.27.14.1. Disease specific genes:

The genes that are known to be involved in Type 2 Diabetes Mellitus were obtained from the "*All gene-disease association database*" from the DisGeneT database v5.0. The common differentially regulated genes between disease phenotype and the *Yoga* and non- *Yoga* groups were obtained. Thirty-Six out of 324 differentially regulated genes were associated with T2DM.

Figure- 42



Enrichment analysis was performed on the T2DM disease specific genes and the results were tabulated. Results suggest that [27202] Complement component 5a receptor 2(C5AR2); [51266] C-type-lectin domain family 1-member B(CLEC1B); [9332] CD163 molecule (CD163); [3554] interleukin 1 receptor type 1(IL1R1) were statistically significantly enriched from amongst the differentially regulated genes.