SECTION - VI

RESULTS

6. Results

All the participants continued with their regular medications till the end of the study period. Of the 60 participants included in the study, 57 participants (26 male and 31 female; age 55.85 ± 9.24; years since diabetes diagnosis, 15.42 ± 7.1; Medication score 1.91 ± 0.27; 28 in intervention and 29 in control group) successfully completed the study. Two participants from intervention group withdrew, due to reasons not related to the study.One participant fell sick with fever before start of the first day of intervention and the other participant could not attend as he was travelling during the intervention week. One participant in the control group could not complete the study as he was asked to remove the sensor while undergoing a medical procedure not relevant to the study. Baseline GV of all three participants was available and used for the intention-to-treat analysis. Table 1 shows the baseline characteristics of both the group including age, HbA1C, duration of diabetes and medication score, and the difference in baseline of both the groups were not statistically significant ($p > 0.05$).

Continuous Glucose Monitoring (CGM) was done from day 1 to day 14. No intervention was administered during the first 7 days and the data was used for establishing baseline for glycemic variability (GV). From day 8 to 14, participants were following either moderate walking for one hour per day (control group) or one hour of yoga per day (intervention group) and the GV post-intervention was measured at the end of the study.

6.1 Daily mean glucose levels

Baseline daily mean glucose levels were 142. 21 mg/dL (± 39.78) and 142.91 mg/dL (±26.34) in control and intervention group respectively, and the difference between

groups at baseline was statistically insignificant. After 7 days intervention the daily mean glucose levels reduced by 23.99 mg/dL (± 18.77) in intervention group, with a statistical significance of $p < 0.001$. Similarly, there was a statistically significant ($p<0.05$) difference of 8.69 mg/dL (±27.08) in the control group as well. However, the difference in daily mean glucose between groups measured using Mann-Whitney U test was not statistically significant ($p>0.05$).

Table 3- Results of statistical tests between control group and intervention group

	Within Group (Control)				Within Group (Intervention)				Between Groups		
	Pre Mean ±SD	**Post Mean ± SD**	**Difference ± SD**	**p value**	**Pre Mean ±SD**	**Post Mean ± SD**	**Difference ± SD**	**p value**	**p Value**	**Cohen's *d***	**Effect size (r)**
Mean Daily glucose	142.21 ± 39.77	133.52 ± 39.55	8.69 ± 27.08	p < 0.05#	142.91 ± 26.33	118.92 ± 28.16	23.99 ± 18.78	p < 0.001*	**p > 0.05 ¶**	0.66	0.31
Standard Deviation	51.42 ± 22.96	46.76 ± 22.45	4.67 ± 7.13	p < 0.05*	36.13 ± 10.91	26.34 ± 11.18	9.79 ± 7.55	p < 0.001*	**P < 0.001****	0.70	0.33
Coefficient of variation	35.47 ± 9.85	34.44 ± 10.70	1.03 ± 2.98	p > 0.05#	25.19 ± 5.84	21.86 ± 6.75	3.33 ± 4.09	p < 0.001*	**P < 0.001¶**	0.64	0.31
LI	1276.61 ± 929.55	1140.43 ± 953.32	136.17 ± 428.66	p > 0.05#	733.13 ± 372.12	466.19 ± 359.98	266.95 ± 210.29	p < 0.001*	**P < 0.001¶**	0.39	0.19
MODD	43.8 ± 20.9	42.64 ± 21.12	1.16 ± 8.08	p > 0.05*	29.46 ± 10.2	21.09 ± 9.92	8.38 ± 6.18	p < 0.001*	**P < 0.001****	1.00	0.45
MAGE	115.20 ± 53.06	109.05 ± 54.08	6.15 ± 13.60	p < 0.05*	77.76 ± 24.11	60.29 ± 31.18	17.47 ± 21.24	p < 0.001#	**P < 0.001¶**	0.64	0.30

*-Paired sample t test; #-Wilcoxon signed rank; **-Independent t test; ¶- Mann-Whitney U-test

Key: LI – Lability Index; MODD – Mean of Daily Differences; MAGE – Mean Amplitude of Glucose Excursions

6.2 Variables of GV

In spite of the between group difference in daily mean glucose levels being statistically non-significant, the difference in glycemic variability between group, including standard deviation (SD), Lability Index (LI), Mean of daily differences (MODD), mean amplitude of glucose excursions (MAGE) and average daily risk ratio (ADDR) were statistically significant ($p < 0.05$).

In control group, a statistically significant reduction in standard deviation of 4.67 (±7.13) ($p<0.05$) was observed. In the intervention group, the reduction was even more prominent at 9.79 (±7.56) with $p<0.001$.

Likewise, in control group, MAGE reduction was 6.15 (±13.6) mg/dL ($p<0.05$), while in the intervention group, a statistically significant ($p<0.001$) reduction of 17.47(±21.25) mg/dL was observed (table 2).

DISCUSSION – VII

DISCUSSION

Discussion

To our knowledge this is the first ever study to assess the effect of yoga on glycemic variability. Increased mean blood glucose levels are predictive of microvascular complications (Stratton et al., 2000), while increased glycemic variability is more predictive of macrovascular complications (Su et al., 2011; Karstoft et al., 2017). In the current study, there was a statistically significant reduction in both mean glucose levels and glycemic variability which implies that yoga might possibly be beneficial in preventing both microvascular and macrovascular complications of T2DM.

There was a statistically significant reduction in the 24-hour mean glucose levels in both control group (-8.69 ± 27.08) and intervention group (-23.99 ± 18.78 mg/dL), with non-significant difference between groups ($p > 0.05$). Interestingly, in spite of a non-significant reduction of mean glucose levels between groups, we observed a statistically significant ($p<0.001$) improvement in multiple measures of glycemic variability, including mean amplitude of glucose excursions (MAGE), standard deviation (SD), percentage coefficient of variation (% CV), mean of daily differences (MODD) and Lability index (LI) in the intervention group when compared to the control group.

Multiple measures of dispersion are necessary for assessing effects of GV, because a reduction in GV need not necessarily mean that all the measures of GV have reduced significantly, as observed in previous studies (Dungan et al., 2013). Each measure of GV has its own uniqueness and significance, and adds value to the findings. Standard deviation (SD) measures the amount of variation or dispersion from the average score and is commonly used as an indicator of glycemic variability, however

SD omits consideration of 'number' of glycemic swings which is captured by MAGE. The coefficient of variation (CV) of blood glucose represents the ratio of SD to mean blood glucose. CV is considered as a more useful measure than SD for GV comparisons between groups with different glucose tolerances, which would be a common factor in community based studies. MAGE represents a marker of intraday GV and is considered as the gold standard of GV measurement. MAGE calculation involves computing the arithmetic mean of differences between consecutive peaks and *nadirs*. MAGE quantifies only major swings in glycemia that are >1 SD of mean glucose levels, but excludes minor deviations. MAGE has been shown to be independent of mean glucose levels and higher MAGE readings are associated with increased glycemic instability, as it reflects both upward and downward. Mean of Daily Differences (MODD) is a measure of interday variability (Kohnert et al., 2009). Lability index (LI) is another measure of GV, which is the sum of square of the differences between two successive glucose measurements divided by the difference in time between measurements. LI is found to be a better GV measure than SD or MAGE in identifying mortality risk (Dungan et al., 2013).

Taborsky et al., (2010) postulates three possible mechanisms through which glucagon secretion is modulated in hypoglycaemia, strongly emphasizing on the involvement of autonomic modulation. First mechanism is by direct stimulation of α-cells, second is through reduced inhibition of α -cells by endogenous insulin and third by direct autonomic innervation of the α –cells in response to hypoglycaemia (Taborsky et al., 1999). A reduction in the increased blood glucose levels in diabetes through a mode of physical activity like yoga might be quite straight forward, especially with a large body of available literature evidence on yoga (Kumar et al., 2016). However, the

role of yoga in correcting low blood glucose levels observed in the current study is something unique and is in line with a previous observation, where Manjunatha et al. (2005) reported that following yogic asanas there was a decrease in the insulin release when glucose level tends to fall and an increase in insulin release when glucose levels raises. The specific property of yoga was attributed to the increase in β-cell sensitivity following yoga (Manjunatha et al., 2005).

We put forth three possible mechanisms through which yoga might possibly help in correcting low blood glucose levels, in order to maintain glucose homeostasis and reduce glycemic variability in our study. One possible mechanism is through secretion of acetylcholine (ACh), one of the key neurotransmitter of parasympathetic nervous system believed to be released during regular yoga practise (Singh et al., 2004). Most parasympathetic axons in the vagus nerves pass and innervate the entire thoracic and abdominal regions including the endocrine pancreas (Rodriguez-Diaz and Caicedo, 2014). *In vivo and in vitro* stimulation of the vagus nerve has little effect on the concentration of insulin during hypoglycaemia, but increases it more and more efficiently as the concentration of plasma glucose increases and moreover, experiments using various cholinergic agents have shown that ACh stimulates glucagon through atropine-sensitive mechanisms (Gilon & Henquin, 2001). Meaning that, there are minimal chances of hypoglycemia due to yoga, as β-cell stimulation via acetylcholine is impaired at levels lower than the normal glucose levels. Interestingly, an unpublished data in one of our previous studies (under review) show that 10 days of regular conventional yoga not only reduced the mean glucose levels in participants with baseline hyperglycemia but also increases mean glucose levels in participants with

baseline hypoglycemia. The observation was one of the first of its kind to be reported and was also the basis of our current hypothesis.

Second, through possible stimulation of glucagon-like peptide-1 (GLP-1) through yoga. GLP-1 is a potent incretin hormone, acting more like a 'master switch' in glucose metabolism by operating via multiple pathways like increasing glucose uptake of muscle and liver, inhibiting glucagon secretion, while promoting insulin secretion in a 'glucose regulated' manner (Cervera et al., 2008). Role of vagus in the regulation of GLP-1 secretion has been clearly demonstrated in animal model. Bilateral sub diaphragmatic vagotomy in conjunction with gut transection and selective hepatic branch vagotomy completely abolishes the fat-induced and exogenous GIP induced GLP-1 release respectively, while stimulation of the distal end of the celiac branch of the sub diaphragmatic vagus nerve significantly increases the release of GLP-1 (Rocca and Brubaker, 1999). The gene encoding the GLP-1, the proglucagon gene has three known sites of expression, namely, α- cells, L cells of large intestine and the nucleus tractus solitarius in the hind brain, which is the nucleus of vagus nerve as well (Kieffer and Habener, 1999). Thus, yoga could possibly correct glycaemic variability through its potential role of secreting GLP-1 as well.

Third, through enhancing paracrine communication between different islet cells. The α- and β- cells works in harmony, responding appropriately to increase or decrease the blood glucose concentrations in order to maintain normoglycaemia. Loss of glucagon secretion in response to insulin or insulin secretagogue induced hypoglycaemia is one of the major issues in diabetes management and is attributed to the lack of essential signals from β- cells (Gaisano et al., 2012). The current available

literature shows that islet cells profoundly modulate each other's secretory functions by very complex cross talk through paracrine and autocrine pathways- stemming from hormones or neurotransmitters like ACh and GABA- which shapes the secretion of insulin and its counter regulatory hormone, glucagon in a glucose dependent manner (Gaisano et al., 2012; Benninger et al., 2018). Yoga through its known effect on modulation of ACh and GABA (Streeter et al., 2010), could thus facilitate better communication between the different islet cells in response to changes in blood glucose concentration, ensures optimum blood glucose levels without swinging towards either hypo- or hyperglycaemia.

It is worth mentioning that the yoga practices administered in our study and the above mentioned study are based on conventional yoga practices (except warming up) mentioned in the traditional scriptures in a slow and synchronised manner. No modern modified yoga forms like power yoga, hatha yoga or hot yoga were used. Power yoga and other modern forms of yoga believe in reducing the calories and thereby reducing bodyweight and insulin resistance, resulting in reduction of blood glucose levels. We have chosen a more conventional form of yoga, because our hypothesis puts forth 'β-cell dysfunction' as the primary defect in T2DM, especially in the context of the Indian population (Staimez et al., 2013). Chandalia et al (2007) have shown that South Asians are more prone to T2DM at a lesser BMI when compared to the Caucasians and have higher β-cell dysfunction than IR (inspite of higher abdominal obesity) (Chandalia et al., 2007; Vijayakumar et al., 2017). This particular thesis is based on one particular aspect in the pathophysiology of β-cell dysfunction, glycaemic variability. And this reduction in variability helps in overcoming the dysfunction by bringing about a balance, as per our yoga definition of interest - '*Samatvam yoga uchyate'*.

SECTION VIII

CONCLUSION

Conclusion

Glycemic variability strongly correlates with β-cell dysfunction and yoga thus might be helpful in better management of in β-cell dysfunction T2DM (Kohnert et al., 2012). The need for an effective and cost-efficient treatment strategy in the secondary prevention of T2DM is increasing. Future implications of yoga in the management of T2DM and preventing complications of T2DM are very promising, with lesser risk of hypoglycaemia and glycemic variability.