Summary and Conclusion

The major objective of this study was to see whether HDL can be glycated by glyoxal and glucose and further whether the glycated HDL would undergo modification from an anti-inflammatory, antiatherogenic molecule to a pro-inflammatory atherogenic molecule. The specific observations are as follows:

- HDL could be glycated by glyoxal with an accompanying loss of PON activity.
- Glucose protected the PON from inactivation even at a relatively high concentration of glucose (500mM).
- Inactivation of PON by glyoxal was dose and time dependent.
- Glucose protected PON from inactivation for up to 7 days of in vitro glycation.
- Glyoxal caused a increase in the thiolactonase activity of HDL at 1M concentration but had no effect at 100mM concentration.
- Epoxidase activity was inhibited by both glyoxal and glucose.
- Tryptophan fluorescence increased by a small but significant amount (p<0.05) after glycation with glucose but not with glyoxal.
- AGE detected by fluorescence increased with glycation. Glyoxal was more effective than glucose.
- Glyoxal and glucose modified amino groups equally at 1M conc. At 100mm concentration both were not very effective.
- Thiol groups were modified by glyoxal at 100mm concentration whereas glucose modified them at 1M concentration.
- Glyoxal increased carbonyl content about 73 fold whereas glucose did not increased the carbonyl content of HDL.
- Esterase activity of HDL was not recovered from Cibacron blue sepharose column chromatography whereas PON activity was recovered without change.
- Only 5% of the total serum activity bound to the column. Remaining 95% did not bind.
Summary and Conclusion

- Glycated HDL did not show any distinct changes in bands or mobility in SDSPAGE. But in native PAGE there was an increase in the mobility.
- 15KD peptide was missing from the MALDI-TOF of glycated albumin. Many proteins known to be present in HDL did not show their presence in mass spectrum, probably because of poor ionizability.
- Glycation of HDL reduced the HDL-C.
- CD spectra of HDL were typical of a protein rich in \( \alpha \)-helical content.
- Glycation with glucose or glyoxal caused similar changes.
- Glycation brought about a change in \( \alpha \)-helical content. \( \alpha \)-helical content decreased and \( \beta \)-sheet content increased.
- Glycation of endothelial cells in the umbilical cord could not be detected in the histology slides with the hematoxyline-eosine.
- But they had intense green fluorescence typical of AGEs.
- Vitamin C was able to protect PON activity from inactivation by glyoxal. But it could not protect glucose catalysed inactivation.
- Vitamin E protected inactivation of PON by glyoxal as well as glucose.
- Quercetin was able to protect PON from inactivation induced by glyoxal, but not glucose.
- ApoA1 when glycated was antigenic to rabbits and hens.
- Antibody with low titre was formed in rabbits and in hen’s egg yolk.
- Rabbit antibody had no cross reactivity but the IgY had cross reactivity.
- Antibody could quantitatively assay the antigen in an ELISA test.
- When glycated ApoA1 was assayed in 30 diabetics and 10 controls, there was no significance difference.
- HDL at higher concentration (>45mg/dl) appear to prevent glycation.
- Glycated ApoA1 and HbA1c showed a linear correlation.
- Antibodies raised to oxidized HDL cross reacted with glycated HDL.
- When HDL was glycated and precipitated with the antibody, glyoxal mediated glycation gave more PON activity in the precipitate.
- Corresponding supernatants had less PON activity.
There was no difference in the DCFH fluorescence of precipitate or supernatant and control.

In a study with diabetic subjects HbA1c increased with duration of diabetics.

Women had higher HbA1c than men

HbA1c did not correlate with Hb content but correlated with duration of diabetes.

Erythrocytes could be glycated invitro. HbA1c so formed increased with the glycating agent.

Plasma glucose and serum glucose linearly correlated. However serum glucose values were 1-15% lower than the corresponding plasma glucose values.

Blood glucose values decreased in plasma and serum on storage.

EDTA plasma gave the lowest values, Fluoride plasma gave the highest values.

Loss of blood glucose in fluoride plasma was the least.

HbA1c did not correlate with esterase activity or paraoxonase activity.

**Overall conclusions**

Our results suggest that non enzymatic glycation of HDL leads to structural and functional modification which results in the loss of HDL-associated PON1. This may cause the loss of anti-oxidant activity of HDL making it a pro-inflammatory molecule and hence pro atherogenic.

**Future perspectives**

The global burden of diabetes is increasing in spite of all the diagnostic and therapeutic advanced that have taken place in the last 20 years. A new insight into the whole picture of diabetes is what is needed now.

Historically, it is believed that Mikowski noticed that pancreactimized dogs urinated more frequently and that the urine attracted a large number of flies. Popular legend states that he tasted the urine and noticed its sweetness (Boden and Laakso 2004) Interestingly Banting and Best were awarded the Nobel Prize for a factor that “abolished” sugar in urine. These two landmark events set the stage for the glucose-
Insulin axis. Mc Garry (Mc Garry 1992) commented that if Minkowsky had smelled the urine of diabetic dogs instead of tasting it, he would have smelled acetone and the directions of research on diabetes would have taken a different route.

While there is an obvious association between obesity, hyperlipidemia and diabetes, the role of dietary sugar cannot be ignored. Purified cane sugar (sucrose) and high fructose syrup consumption has increased globally and the trends parallel the increase in incidence of diabetes.

Apart from pancreas the organ which has maximum role is the adipose tissue while 80% of the adipose tissue (Arner 2001) is subcutaneous, about 10% is visceral. The visceral adipose tissue is not only responsible for the diabetes related complications but also for other disorders like cardiovascular diseases. Adipocytes are also hormonally active and produce a variety of hormones. Adiponectin is one such hormone which is associated with the increased fatty acid oxidation and increased insulin sensitivity (Yamanchi et al 2001). This may explain why some obese people do not develop diabetes or cardiovascular diseases.

Fructose – induced lipogenesis however may probably not come under the control of glucose regulations since fructose is not responsive to insulin. The VLDL synthesized through fructose ingestion can in fact clog small arterioles resulting in diabetic peripheral neuropathy, and other microvascular complications.

So should we be looking for one more drugs for diabetes? Or should we take a different prospective? Since non enzymatic glycation has been attributed as the cause of diabetic complications, inhibition of AGE formation and its subsequent interaction with RAGE may be an approach for the future.

AGE have one more effect namely increasing reactive oxygen species (ROS) (kawahito et al 2009). ROS has been shown to inhibit pancreatic duodenal homeobox-1(PDX-1) which is a transcription factor for insulin gene (Kaneto et al 2001). Thus oxidative stress is the underlying causes for the decreased insulin secretion I the pancreas in diabetes. CVD and cancer are recognized as diseases associated with increased oxidative stress. However, antioxidant therapy has not been effective in any of these three diseases in evidence based medicine.
So what is the way forward? From studies that have been carried out in the recent past, there is overwhelming evidence against consumption of pure sugars. Natural sugar substitutes may be the answer in satisfying the sweet taste but without the added complication of sugars.

Gut microflora may also be equally important in our well-being particularly in relation of CVD and perhaps even diabetes. Analysis of gut microflora of normal healthy individuals and those with chronic hyperglycemic may provide clues to controlling diabetes without the intervention of drugs.