Research Paper

ALCOHOL INDUCED LIPID DYS-HOMEOSTASIS IN THE PREFRONTAL CORTEX OF DIABETIC RATS

ABSTRACT

Aim: To investigate the potential importance of the association between alcohol consumption, diabetes andlipid homeostasisin the prefrontal cortex.

Study Design: Twenty-four adult rats were randomly divided into four groups of six rats each viz; Group 1- Control, given rat pellets and water *ad libitum*; Group 2- Diabetic, diabetes was induced with a single dosage of 120mg/kg body weight followed by 50mg/kg body weight of alloxan weekly. Group 3- Diabetic and low alcohol intake (9% w/w). Group 4- Diabetic and high alcohol intake (20% w/w).

Place and Duration of Study: This work was carried out in the Department of Anatomy; Olabisi Onabanjo University, Ago-Iwoye, Nigeria between February and April, 2012.

Methodology: The skull was dissected and the brain removed and the prefrontal cortex excised, homogenized, centrifuged and the supernatant analyzed for lipid profile, AST and ALT.

Results: Diabetes elevates the levels of ALT and AST significantly at P=0.05 when compared to the normal control group (67.33+/-0.15 & 59.25+/-0.25 and 135.50±0.50 & 75.50+/-0.50) respectively. The TG levelsincreasesignificantly at P=0.05 in the diabetic group compared to the normal control group (92.01+/-0.18 versus 72.62+/-0.52) whereas the total cholesterol and HDL levels decrease in the diabetic group when compared to normal control group (34.99+/-0.40 &

37.28+/-0.23 and 150.90+/-0.33 & 103.29+/-0.23 respectively). Alcohol intake on the other hand lowered the levels of all parameters significantly at *P*=0.05.

Conclusion: Our findings showed that, both low and high chronic alcohol intake in diabetes disturbed lipid homeostasis in the prefrontal cortex, probably by lowering ALT and AST levels or via the mechanism that suppresses the enzymes of lipid syntheses in the prefrontal cortex.

Keywords: Diabetes; Alcohol; Lipids; Prefrontal cortex

1.0 Introduction

Numerous studies have demonstrated a J-shaped relationship between alcohol consumption and diabetes [1, 2]. Moderate consumption of alcohol is medically beneficial and socially acceptable [3], as moderate alcohol consumption also beneficially affects insulin sensitivity and glucose metabolism [4]. However, excessive consumption of alcohol leads to complex health problems, because alcohol may alter glycemic regulation and potentially aggravate the cardiovascular, neurological, and immunosuppressive changes in patients with diabetes mellitus. Because alcohol use, at least on a social level, is widespread among diabetics as well as non-diabetics, clinicians and researchers must understand effects of alcohol on the progression and complications of diabetes [5]. It is known that chronic alcoholics and type 2 diabetics show hyperlipidemia, characterized by hypertriglyceridemia and in a minor degree by hypercholesterolemia [6]. The most typical lipoprotein pattern in diabetes, also known as diabetic dyslipidemia or atherogenic dyslipidemia, consists of moderate elevation in triglyceride and low HDL cholesterol values, and increase in small dense LDL particles. This lipoprotein pattern is associated with insulin resistance and is present even before the onset of diabetes. LDL cholesterol levels in type 2 diabetic subjects are generally similar to those found in the general population. Small dense LDL particles are highly atherogenic because of their enhanced

susceptibility to oxidative modification and increased uptake by the arterial wall [7].The mechanisms underlying the effect of ethanol and carbohydrates on plasma lipids seem to be different; therefore in diabetic subjects chronic alcohol consumption could produce a more severe hyperlipidemia and so accelerate atherosclerotic events [6]. There are limited reports on the effect of alcohol during diabetic condition with reference to total cholesterol, triglycerides, high density lipoprotein in brain tissue. Hence this study investigated the potential importance of the association between alcohol consumption, diabetes and lipid dys-homeostasisin the prefrontal cortex of the brain.

2.0 Materials and Methods

2.1 Experimental Animals

Twenty-fourwistar rats, weighing (150-170gm) were used for this study. The animals were housed in clean plastic cages, well ventilated environment with temperature ranging between 24-28ºC in 12 hours light and 12 hours dark cycle. The animals were given standard rat pellets and water *ad libitum*, and were allowed to acclimatize for four weeks before commencing the experimental protocols. The institutional committee on Animal Care and Use in Research, Education and Testing (ACURET) approval was sort for and the animal experiments were conducted according to the NIH Guide on Laboratory Animals for Biomedical Research (NIH, 1978) and ethical guidelines for investigation of experimental pain in conscious animals [8].

2.2 Experimental Design

A total of twenty-four rats were randomly divided into four groups of six rats viz;

Group 1- Control, given normal rat pellets *ad libitum* and water

Group 2-Diabetic, diabetes was induced with multiple dosages of 120mg/kg body weight of alloxan for a start and subsequently 50mg/kg body weight weekly till the end of the experiment.

Group3- Diabetic and low alcohol consumption, the rats in this group received multiple dosages of alloxan (as in group 2). After establishing diabetes, they were given 9% w/w alcohol daily.

Group 4- Diabetic and high alcohol consumption, the rats received multiple dosages of alcohol (as in group 2), they were given 20% w/w alcohol.

Fasting blood glucose level and body weight of the animals were monitored regularly throughout the duration of the experiment which lasted four weeks.

2.3 Tissue sample preparation

At the end of four weeks the rats were euthanized by administering 10g/kg body weight of pentobarbital. The rats were then dissected and the skull cut open and a portion of the prefrontal cortex excised homogenized and centrifuge at 3500rpm for 15minutes. The supernatant was collected for biochemical analyses.

2.4 Biochemical Analyses

Aspartate Aminotransferase-AST and Alanine Aminotransferase-ALT were both determined by the method of Reitman and Frankel, [9] using Randox Reagent Kits. Determination Triglycerides (GPO method) by Fossati et al. [10], Total Cholesterol by Allain et al. [11], High Density Lipoprotein (HDL) – Cholesterol (PTA) by Tietz [12].

2.5 Statistical Analysis

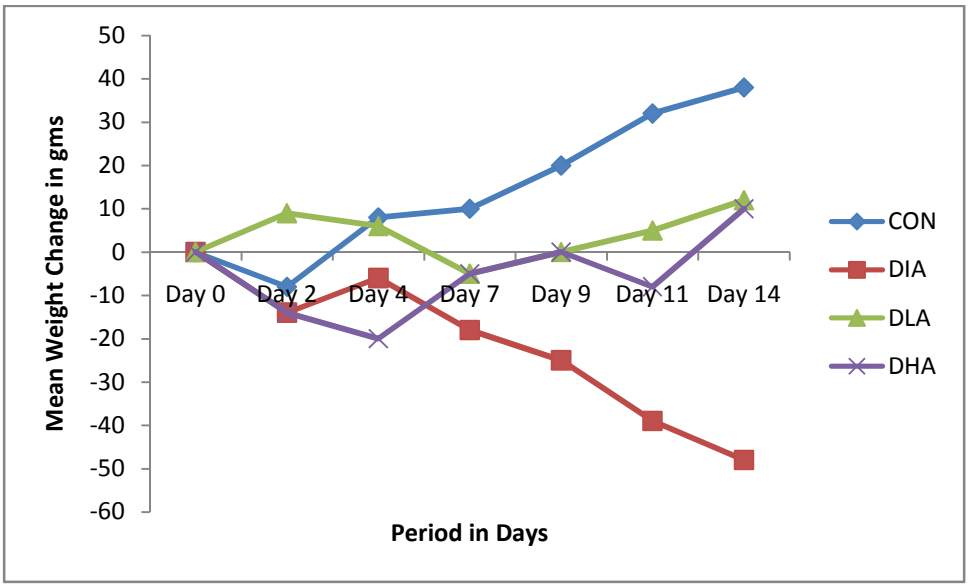
Data were analysed by comparing values for different treatment groups with the values for individual controls. Results were expressed as mean ± SEM. The significant differences among values were analysed using SPSS version 19 at *P-value* = 0.05.

3.0 Results

3.1 Mean body weight in the experimental group

The body weights of control group dropped initially and then increase steadily, while the diabetic group witnessed a consistent drop. Both high and low chronic alcohol intakes improve body weight in diabetic rats. (Figure 1)

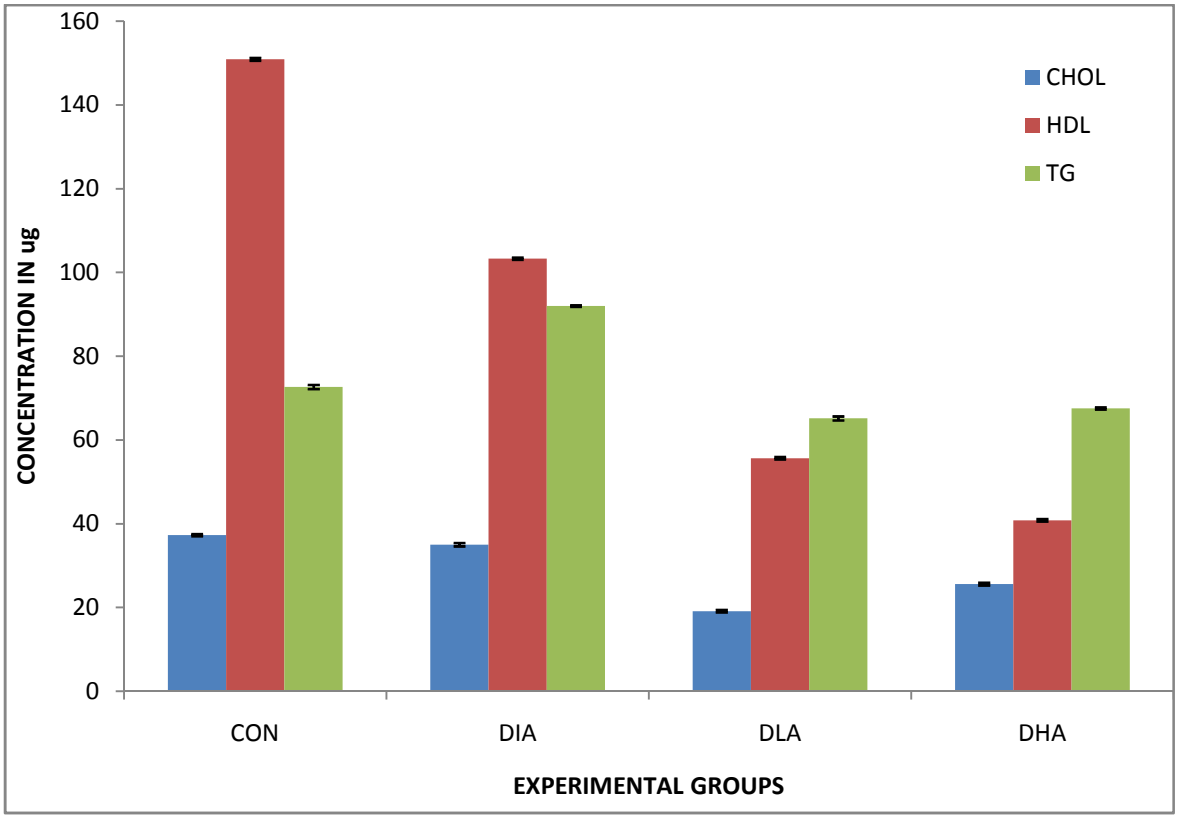
Figure 1: mean weight change in experimental groups (CON=Control; DIA=Diabetic; DLA=Diabetic and Low Alcohol; DHA=Diabetes and High Alcohol)



3.2 Mean level of Triglycerides, total cholesterol and high density lipoprotein in the prefrontal cortex

Both diabetes and alcohol lowered high density lipoprotein (HDL) total cholesterol significantly at (P= 0.05), while triglycerides level was significantly increased by diabetes. Alcohol intake exacerbates the effect of diabetes. (Figure 2)

Figure 2: lipid concentration in the prefrontal cortex of CON=Control; DIA=Diabetic; DLA=Diabetic and Low Alcohol; DHA=Diabetes and High Alcohol



Diabetes increased significantly (P< 0.05) the level of triglycerides and significantly (P< 0.05) lowered the total cholesterol and high density lipoprotein in the prefrontal cortex; while alcohol intake lowered the levelsin all groups.. (Table 1)

Table 1: Mean levels of triglycerides, cholesterol and high density lipoprotein in the prefrontal cortex

GROUPS	TRIGLYCERIDES		CHOLESTEROL		HIGH DENSITY LIPOPROTEIN	
Mean & S.E	**p-value**	**Mean & S.E**	**p-value**	**Mean & S.E**	**p-value**	
CONTROL	72.62±0.52	0.002	37.28± 0.23	0.169 x	150.90± 0.33	0.008
DIABETIC	92.01±0.18		34.99± 0.40		103.29± 0.26	
CONTROL	72.62±0.52	0.059	37.28± 0.23	0.003	150.90± 0.33	0.002
DLA	65.14±0.43		19.10± 0.30		55.66± 0.27	
CONTROL	72.62±0.52	0.014	37.28± 0.23	0.006	150.90± 0.33	0.001
DHA	67.53±0.22		25.56± 0.32		40.83± 0.30	
DIABETIC	92.01±0.18	0.015	34.99± 0.40	0.028	103.29± 0.26	0.003
DLA	65.14±0.43		19.10± 0.30		55.66±0.27	
DIABETIC	92.01±0.18	0.001	34.99± 0.40	0.034	103.29± 0.26	0.008
DHA	67.53±0.22		24.56± 0.32		40.83± 0.30	
DLA	65.14±0.43	0.152 x	19.10± 0.30	0.018	55.66± 0.27	0.021
DHA	67.53±0.22		25.56± 0.32		40.83± 0.30	

P value is significant at p=0.05 **Not Significant=** x

Diabetes increased significantly (P< 0.05) the levels ALT and AST while alcohol intake in diabetics lowered the level of ALT and AST significantly(P< 0.05) in the low and high intake respectively. (Table 2)

Table 2: Mean levels of Alanine aminotransferase (ALT) and Aspartate transaminase (AST)

GROUP	Alanine aminotransferase (ALT)		Aspartate transaminase (AST)	
Mean & SE	***p*-value**	**Mean & SE**	***p*-value**	
CONTROL	59.25± 0.25	0.008	75.50± 0.50	0.011
DIABETIC	67.33±0.15		135.50± 0.50	
CONTROL	59.25±0.25	0.002	75.50± 0.50	0.019
DLA	24.48±0.28		73.85±1.35	
CONTROL	59.25±0.25	0.005	75.50± 0.50	0.006
DHA	26.63±0.18		60.25±0.25	
DIABETIC	67.33±0.15	0.001	135.50±0.50	0.013
DLA	24.48±0.28		73.85±1.35	
DIABETIC	67.33±0.15	0.008	135.50±0.50	0.010
DHA	26.63±0.18		60.25±0.25	
DLA	24.48±0.28	0.133 [x]	73.85±1.35	0.051
DHA	26.63±0.18		60.25±0.25	

P value is significant at p=0.05 **Not Significant=** [x]

4.0 Discussion

Diabetes mellitus is characterized by hyperglycemia together with biochemical alterations of glucose and lipid peroxidation [13] [14]. The mechanisms underlying the effect of ethanol and carbohydrates on plasma lipids seem to be different. In the present study diabetes was characterized by significant hypertriglycerides and hypocholesterol in the prefrontal cortex while alcohol ingestion in diabetic rats significantly lowered brain tissue triglycerides and cholesterol.

In hyperglycemic states, there will be intracellular glycogen accumulation in the hepatocytes due to increased glycogen synthesis, causing typical biochemical findings of mild to moderately elevated aminotransferases as reported in this study by the significant elevation in the levels of ALT and AST in diabetic rats due to tissue compromise and increased physiological needs. ALT, produced mainly in the liver, catalyzes the transfer of amino groups between L-alanine and glutamate to meet physiological needs. AST catalyzes the transfer of amino and keto groups between alpha-amino acids and alpha-keto acids thereby acquiring the term transferase [15]. Alcohol seems to not only reverse this effect but lower the levels below the control level probably by immuno-depletion of ALT1 which decreases majority of the serum ALT activity in healthy humans [16].

The finding of hypertriglyceridemia in the diabetic group is consistent with the previous reports [17,18,19].The increase could be due to the increase in lipid peroxidation, a free radical-related process which is an uncontrolled, self-enhancing process causing disruption of membranes, lipids and other cell components. Unlimited lipid peroxidation (LP) could be one of the main factors in the pathogenesis of diabetic complications. This pathology is often related to the release of free radicals which cause oxidative stress [20]. During reoxygenation, hypoxantine-xantine oxidase and arachidonic acid pathways are important sources of free oxygen radicals,

which damage lipid membranes, and lead to cytolysis and cell death [21]. Accumulation of lipids in diabetes is mediated through a variety of derangements in metabolic and regulatory processes, especially insulin deficiency, thereby rendering the diabetic patient more prone to hypertriglyceridemia.

Our results also showed that the total cholesterol level was significantly decreased in the prefrontal cortex. The brain being the most cholesterol-rich organ in the body, most of which comes from in situ synthesis [22]. Brain cholesterol turnover is increased in Alzheimer's and other neurodegenerative diseases, and has been suggested to play a role in pathogenesis of these disorders [23]. Diabetes mellitus is associated with a variety of neurologic and cerebral complications, including cognitive dysfunction, depression, and increased risk of Alzheimer's disease [24, 25, and 26]. The hypocholesterolemia reported in this study could be explained by the high insulin levels caused by diabetes which impair insulin signaling in the brain and affect lipid metabolism [27]. It is also characterized by decreased sensitivity and resistance to insulin [28].Previous report indicates that chronic alcohol abuse causes a Type 2 diabetes effect in certain brain regions. Investigations on human tissue show that high levels of chronic alcohol consumption lowers the levels of genes needed to respond to insulin and insulin growth factor (IGF) resulting in brain damage[28]. Apart from effects on lipid metabolism, haemostatic balance and blood pressure, alcohol improves insulin sensitivity. This improvement of insulin sensitivity may also be responsible for the lower incidence of type 2 diabetes mellitus reported to be associated with light-to-moderate drinking [29]. Ethanol-induced perturbations in membrane lipid composition contribute to insulin/IGF resistance in brain. One possible explanation is that, besides cholesterol, ethanol depletes a variety of membrane lipids [30]. In this study, diabetes and alcohol seemed to have a common pathway for the down regulation lipid biosynthesis

pathway in the prefrontal cortex of treated rats. These have been associated with a change in gene expression which leads to a decrease in cholesterol synthesis in brain and a decrease in cholesterol content in synaptosomal membranes. Mimicking the reduction in cholesterologenic genes in cultured neurons by knockdown of SREBP-2 results in reduced markers of synapse formation, and knockdown of SREBP-2 in the hypothalamus in vivo causes altered feeding behavior and dysregulation of counter insulin hormones [31]**.** Over the past decade there have been important advances in defining the mechanisms that cells use to regulate cholesterol levels and lipid metabolism [32], includinga strong feedback system in which cholesterol and sterol intermediates regulate the activation of the SREBP family of transcription factors. While it is likely that this feedback system is active in brain, there is a failure to compensate for reduced cholesterol synthesis and reduced synaptosomal cholesterol with up-regulation of cholesterologenic genes in diabetes. This might be due to morphological complexity of neurons and astrocytes that have long processes spatially distant from the endoplasmic reticulum, where SREBP cleavage-activating protein (SCAP) works as a sensor, or might represent a fundamental difference in regulation of cholesterol synthesis in brain and liver[33].

CONCLUSION

Our findings showed that, both low and high chronic alcohol intake in diabetes lead to lipid dyshomeostasisin the prefrontal cortex, probably by lowering ALT and AST levels or via the mechanism that suppresses the enzymes of lipid syntheses in the prefrontal cortex, leading to reduction in synaptosomal membrane cholesterol, and altered neuronal and physiological function.

CONSENT

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist

References

1. HoffmeisterH, SchelpFP, MensinkGB, Dietz E, BohningD. The relationship between alcohol consumption, health indicators and mortality in the german population.Int J Epidemiol. 1999; 28:1066–72.

2. Dawson DA. Alcohol, alcohol dependence, and all-cause mortality.AlcoholClinExp Res. 2000; 24:72–81.

3. Klastsky AL, Armstrong MA, Friedman GD. Alcohol and mortality. Ann Intern Med. 1992; 117:646–54

4. Facchini F, Chen YD, Reaven GM. Light-to-moderate alcohol intake is associated with enhanced insulin sensitivity. Diabetes Care.1994; 17:115–9.

5. Nicholas V. Emanuele, Terrence F. Swade, and Mary Ann Emanuele.Consequences of Alcohol Use in Diabetics.Alcohol Health & Research World.1998; Vol. 22, No. 3

6. Bertello PD, Gurioli LZ, Gurioli LR, Magro E. Effect of chronic alcohol consumption on blood lipid levels and angiopathy in diabetics].Minerva Med. 1989; 80(5):443-6.

7. James Best, George Jerums, Harvey Newnham, Richard O'Brien. Diabetic Dyslipidemia.Dept Medicine, St Vincent's Hospital; Endocrine Unit, Austin Hospital; Dept Medicine, Box Hill Hospital; Diabetes Unit, Monash Medical Centre, Victori ADS Position Statements. 1994

8. Zimmermann M: Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983; 16:109–110

9. Retimen S, Frankel SA. Colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvate transaminases.Am J ClinPathol. 1957;28:56-63

10. Fossati P, Prencipe L. Serum Triglycerides Determined Colorimetrically with an Enzyme That Produces Hydrogen Peroxide. Clin. Chem. 1982;28(10):2077-80 .

11. Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC. Enzymatic Determination of Total Serum Cholesterol.Clin. Chem. 1974;20(4):470-5.

12. Tietz NW. Text book of Clinical chemistry (3rd Ed.) (eds, C.A Burtis, E.R Ashwood,W.B Saunders); 1999;819-861.

13. Pari L, Latha M. Effect of Cassia auriculata flowers on blood sugar levels, serum and tissue lipids in streptozotocin diabetic rats. Singapore Med J. 2002; 43:617–21.

14. Nordrnann R. Alcohol and antioxidant systems. Alcohol Alcohol.1994; l29:513–22.

15. Han Ni, HtooHtooKyawSoe, AungHtet**.** Determinants of Abnormal Liver Function Tests in Diabetes Patients in Myanmar. International Journal of Diabetes Research 2012, 1(3): 36-41

16. Lindblom P, Rafter I, Copley C, Andersson U, Hedberg JJ, Berg AL, Samuelsson A, et al. Isoforms of alanine aminotransferases in human tissues and serum--differential tissue expression using novel antibodies. Arch BiochemBiophys. 2007; 466:66–77.

17. Palanduz S, Ademoglu E, Gokkusu C, Tamer S. Plasma antioxidants and type 2 diabetes mellitus. Res CommunMolPatholPharmacol.2001; 109:309–18.

18. Warso MA, Lands WE. Lipid peroxidation in relation to prostacyclin and thromboxane physiology and pathophysiology.Br Med Bull. 1983;39:277–80.

19. Montenegro RM Jr, Montenegro AP, Fernandes MI, de Moraes RR, Elias J Jr, Gouveia LM, Muglia VF, Foss MC, Moreira AC, Martinelli CE Jr. Triglyceride-induced diabetes mellitus in congenital generalized lipodystrophy. .J PediatrEndocrinolMetab.2002; 15(4):441-7.

20. Zilkens, R. Hypertension: Journal of the American Heart Association. 2005; vol 45: pp 1-6.).

21. Sapna Smith and Alok M Lall.A Study on Lipid Profile Levels of Diabetics and Non-Diabetics AmongNaini Region of Allahabad, India.Turkish Journal of Biochemistry–Turk J Biochem. 2008; 33 (4); 138–141.

22. Dietschy, J.M., and Turley, S.D. Cholesterol metabolism in the brain. Curr.Opin. Lipidol. 2001; 12, 105–112.

23. Reiss, A.B., Siller, K.A., Rahman, M.M., Chan, E.S., Ghiso, J., and de Leon, M.J. Cholesterol in neurologic disorders of the elderly: stroke and Alzheimer's disease. Neurobiol.Aging. 2004; 25, 977–989.

24. Biessels, G.J., Deary, I.J., and Ryan, C.M. Cognition and diabetes: a lifespan perspective. Lancet Neurol. 2008; 7, 184–190.

25. Craft, S., and Watson, G.S. Insulin and neurodegenerative disease: shared and specific mechanisms. Lancet Neurol. 2004; 3, 169–178.

26. Cukierman, T., Gerstein, H.C., and Williamson, J.D. Cognitive decline and dementia in diabetes—systematic overview of prospective observational studies. Diabetologia.2005; 48, 2460–2469.

27. Buettner C; Impaired brain insulin signaling affects lipids, lifespan, and reproductive fertility. Buettner Laboratory for Metabolism and Diabetes. Mount Sinai school of Medicine

28. Van de wiel. Diabetes mellitus and alcohol.DiabetesMetab Res Rev. 2004; 20(4):263-7

29. Ryo Suzuki, Kevin Lee, EnxuanJing,Sudha B. Biddinger, Jeffrey G. McDonald, Thomas J. Montine, Suzanne Craft,andC. Ronald Kahn. Diabetes and Insulin in Regulation of Brain Cholesterol Metabolism.Cell Metab.2010; 12(6): 567–579.

30. Suzanne M. de la MonteLisa Longato, Ming Tong, Sarah DeNucci,and Jack R. Wands. The Liver-Brain Axis of Alcohol-Mediated Neurodegeneration: Role of Toxic Lipids. Int J Environ Res Public Health. 2009; 6(7): 2055–2075.

31. Daniel H. Rasolt. Alcohol Induced Brain Damage Parallels Type 2 Diabetes. Defeat Diabetes Foundation, Inc Posted: 2008, Sunday, June 22.

32. Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the completeprogramofcholesterol and fatty acid synthesis in the liver. J Clin Invest. 2002; 109:1125–1131

33. Ryo Suzuki, Kevin Lee, Enxuan Jing, Sudha B. Biddinger, Jeffrey G. McDonald, Thomas J. Montine, Suzanne Craft, and C. Ronald Kahn. Diabetes and Insulin in Regulation of Brain Cholesterol Metabolism.Cell Metab. 2010; 12(6): 567–579