CHAPTER 4

Discussion and Conclusion

In recent years, the prevalence of NAFLD in young adults has been increasing at an alarming rate that parallels the same tendency taking place around the world. The prevalence of obesity in young adults is double that of younger ages [4]. Various researchers have examined the close relationships that exist between obesity, dyslipidaemia, insulin resistance, and NAFLD [53, 54]. MRI has proven to be powerful imaging tools for liver cirrhosis diagnosis [55], and is known for its ability to quantify the levels of liver fat non-invasively and accurately using ^{1}H MRS technique. This technique is also suitable for longitudinal follow-up when compare to liver biopsies.

The results of this study confirmed once again the association between BMI and LFC, the higher risk of dyslipidaemia, the probability of insulin resistance, and the prospect of metabolic disease in young adults. The highlight of this study is that LFC in the OW/OB group is almost 3 times higher when compared to control group, even if both group were revealed to be healthy. The results showed that 48.7% of OW/OB group are considered to have NAFLD (LFC >5.56%) with no previous diagnosis of chronic liver disease. This rate of prevalence is consistent with earlier findings where 57.4% of NAFLD subjects in young adults also had high BMI [8, 56]. This tendency also should be considered along with higher prevalence of dyslipidemia, pre-diabetes, and hyperglycemia among subjects with LFC>5.56 %. Previous studies have demonstrated that dyslipidaemia risks start to rise progressively with the BMIs at 21 kg/m^2, as LDL and Tri levels are used to evaluate the risk for coronary artery disease rises [57].

A new important finding is that the biochemical and anthropographic markers associated with LFC are significantly different between OW/OB and control group. Among the blood lipid markers, Tri and LDL were found to be statistically higher, and HDL was found to be statistically lower when compare to control group. However, no significant

differences were found for Cho, even if the Cho in OW/OB had increased slightly, with almost half of control group having dyslipidaemia. This could be due to the fact that the two characteristics of subjects in this age group were that they were exposed to high caloric, low fiber 'ready-to-eat' foods, consumed sugary beverages, and had low physical activity. These combined effects could positively affect the Cho levels in blood [3, 12].

The Pearson correlation analysis showed moderate correlation of BMI and LFC ($r=0.531$, $p<0.001$) and mild correlation with W/H ratio ($r=0.388$, $p<0.001$) and WC ($r=0.259$, $p=0.022$). The association of BMI and LFC was additionally confirmed by multicollinearity regression analysis as a significant independent variable after being adjusted for age, sex and other anthropometric variables. These outcomes were dissimilar to previous studies that proposed that W/H ratio can be used as a tool to predict the risks of liver cirrhosis and NAFLD in place of BMI [58, 59]. A possible explanation is the difference in fat accumulation mechanisms, and that gaining weight is the main pathogenic mechanism of liver fat accumulation in this age group, as was previously proposed by VanWagner *et al* [60].

HbA1c and FG is also found to be statistically different when compared to the control, with a weak positive correlation taking place with LFC. However, only HbA1c is a statistically significant independent variable for LFC after adjusting for age and sex. This result may suggest that HbA1c is a better tool for revealing the NAFLD effects on insulin resistance than the FG. This assumption is reflected in other research done on the association between HbA1c and NALFD in non-diabetic subjects [61], and on the association of prediabetic characteristics independent of total body fat in obese adolescent with high liver fat assessment by MRI [53]. Elevated HbA1c further confirms the high risk of cardio vascular disease and insulin resistance in overweight and obese young adults.

Results from NMR metabolite analysis shows elevated lipids and glucose levels occurring in the OW/OB group. In the OW/OB group CH_3 lipids, CH_2 lipids, $C\underline{H}_2$-CH= bound of lipids, and total lipid are statistically significantly higher when compared to control group. However, PLS-DA analysis determined that only CH_2 lipids and CH_3 lipids were potential biomarkers. Glucose and the other metabolites are not found to be statistically significant between groups. The increased serum lipids which are aroused from LDL and

VLDL is consistent with the biochemistry analysis from venous blood that also demonstrate significantly elevated LDL content in OW/OB group. This increase of lipids parallels that of previous studies of lipoprotein profiles that show the correlation of NAFLD scores and higher VLDL content both in size and amount as shown from NMR studies [62]. Additionally, previous studies have shown the association between hepatic steatosis with elevated VLDL numbers and high small LDL concentration in adolescents. VLDL is the lipid that is responsible for balancing lipid levels in the liver and blood. Triglyceride accumulation in the liver and insulin resistance will activate VLDL overproduction. High VLDL released from the liver will result in higher LDLs from the pathway, and from triglycerides and cholesterol ester exchanges taking place between VLDL and LDL via the CETP-mediated pathway. Moreover, LDL is a well-known atherogenic factor that contributes to higher risk of cardio vascular disease in NAFLD patients.

The alteration of other metabolites was not significantly different but is nonetheless noteworthy. These changes of metabolite level may reveal the mechanisms underlying NAFLD progression. Choline is an essential nutrient for maintaining cells and the mitochondria membrane. Various studies have found that choline is associated with obesity and NAFLD. Furthermore, choline also can be phosphorylated to phosphatidylcholine which is an important metabolite for transporting LDL, and serves as an intermediary to maintain a balance between fat in the liver and plasma. The alteration of choline in OW/OB group may suggest a modification of lipid metabolism taking place [63, 64].

Higher total glucose (α and β glucose) in OW/OB group is also noted. This result is similar to the increasing serum glucose found in cirrhosis studies [65]. Elevated lactate was also found in this study, which are metabolites that are associated with the gluconeogenesis process. Metabolomics studies on obese subjects have found higher levels of lactate in the blood. This may indicate an increased anaerobic metabolism and glucose production as lactate is a precursor of gluconeogenesis [66]. A previous study has found lactate to be associated with chronic liver cirrhosis. The combination of evaluated levels of both glucose and lactate in serum may be a sign of pyruvate-dehydrogenase pathway and liver function impairment in obese youth adults. In

accordance with present results, previous research suggested the connection of changes taking place in the pyruvate-dehydrogenase pathways with the severity of chronic liver cirrhosis [65]. However, Alanine level remained stable in both control group and OW/OB group, and also did so in this present study.

This result suggests that the lipid level disturbance is not the sole cause for lipid accumulation in the liver but rather the contributor and consequence of fatty liver alongside with various other factors, such as insulin resistance, adipose tissue dysfunction, and endothelial cells.

This study has a few limitations such as the high prevalence of dyslipidemia in the control group that maybe caused by sample characteristics. This group was mostly composed of young adults engaged in academic studies whose exercise levels was determined by questionnaire. There may have been potential for over reporting by the subject. A second limitation is that LDL was calculated by an adjustable ratio equation and was not measured directly by biochemical assessment.

However, to best of our knowledge, this is the first study on the topic of non-invasive assessment of LFC by ^{1}H MRS technique in healthy young adults without any complications or prior diagnosis of chronic disease. The high prevalence of NAFLD (LFC>5.56%) contribute to silent chronic diseases in young adults that had become obese. Although the current study is based on small sample of subjects, the findings have drawn together various interesting subjects on the effects of BMI and how it contributed to LFC. Furthermore, it shows how LFC is a high-risk factor of metabolic syndrome in young adults. Previous studies of young adults after a 39 years follow-up has shown that obesity occurring in young adults is sustained into adult life, thereby increasing their risk of developing severe liver disease [56].

The NMR results has found various alterations of serum metabolites taking place between the control group and OW/OB group. Significantly different total lipid contents, lipids CH_3 (LDL+VLDL), lipids CH_2 (LDL+VLDL), and C<u>H</u>-CH_2= bond of lipid were evident. The PLS-DA analysis also reveals lipids CH_2 (LDL+VLDL) and lipids CH_3 (LDL+VLDL) as the metabolites of importance that affect and distinguish OW/OB group from control group. This outcome may suggest that increased BMIs in young adults have

significant effects on blood lipids, and may be associated with the prevalence of high LFC occurring among the OW/OB group. Therefore, blood lipid evaluations in individuals with high BMI could be the key to detecting the important metabolites found in LFC accumulation mechanisms, and could be a suitable method of risk assessment for NAFLD in OW/OB young adults.

This study suggests that BMI contributes to increasing LFC, and is a possible factor for higher NAFLD risks in overweight and obese young adults. The importance of weight control as the primary means of prevention and control of NAFLD, as well as for the many metabolic syndromes has been proposed. However, this study may reveal the importance in raising awareness for early prevention before NAFLD transitions into chronic liver disease later in adulthood. Future studies on this topic are therefore recommended as young adults are at a high risk for developing severe liver disease. Furthermore, implications of these findings may be forthcoming in future research using longitudinal studies with larger groups of subjects.

In conclusion, it is proposed that the prevalence of high LFC in OW/OB group can be the result of weight gain and obesity, and maybe a leading pathogenic mechanism of liver fat accumulation in young adults. This current study demonstrated the importance of BMI as tools for prevention and control of NAFLD and metabolic syndromes in young adults