INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a clinicopathologic entity defined as presence of hepatic steatosis in individuals who drink little or no alcohol and represents a spectrum of liver disease ranging from bland steatosis to nonalcoholic steatohepatitis (NASH), which is a progressive form of liver disease that may lead to advanced fibrosis, cirrhosis and hepatocellular carcinoma in a subset of affected individuals. The incidence of NAFLD is underreported and varies widely. Nonalcoholic steatohepatitis was found in 3% of Canadians which is similar to that found in western population (2-3%). A study reported a much higher prevalence of NASH in Greeks (40%) as compared with other earlier mentioned population studies, which the authors attribute to deceased physical activity and alterations in dietary habits in Greek populations over recent decades.

There is accumulating evidence supporting an association between NAFLD and metabolic syndrome (MetS). Metabolic syndrome is a disease composed of different risk factors such as obesity, type 2 diabetes or dyslipidemia. The prevalence of this syndrome is increasing worldwide in parallel with the rise in obesity. Indeed, NAFLD is recognized as the liver manifestation of MetS. Insulin resistance is increasingly recognized as a key factor linking MetS and NAFLD. Insulin resistance is associated with excessive fat
accumulation in ectopic tissues, such as the liver, and increased circulating free fatty acids, which can further promote inflammation and endoplasmic reticulum stress. This in turn aggravates and maintains the insulin resistant state, constituting a vicious cycle. Importantly, evidence shows that most of the patients developing NAFLD present at least one of the MetS traits[9]. Pathological findings in NASH include macrovesicular steatosis, ballooning degeneration (with or without Mallory bodies), with lobular or portal inflammation, (with or without fibrosis)[9]. Careful histologic examination of the liver biopsy by an expert pathologist is required, in an appropriate clinical context for proper diagnosis of NASH. The aim of this manuscript is to review the pathophysiology diagnosis, and management of NASH.

NASH PATHOPHYSIOLOGY

The understanding of the pathophysiology of NASH is still incomplete and currently evolving. Two English physicians described the “two-hit” theory of NASH pathogenesis more than a decade ago. They mentioned that the first step in the development of NASH was the build-up of fat in the liver, while in the second step this fat causes oxidant stress and liver injury[10]. Unfortunately, this theory has not been proven, nor has it provided the basis for any effective therapy. A multiple parallel hit hypothesis was suggested by Tilg and Moschen. They stated that inflammation might precede steatosis in some instances. Therefore, NASH could reflect a disease where inflammation is followed by steatosis. In contrast, NASH subsequent to simple steatosis may be the consequence of a failure of antilipotoxic protection. In both situations, many parallel hits derived from the gut and/or the adipose tissue may promote liver inflammation. Endoplasmic reticulum stress and related signaling networks, (adipo) cytokines, and innate immunity are emerging as central pathways that regulate key features of NASH[9].

The use of antioxidants in treating the resultant liver injury have shown no promising results, regardless of possible fat build-up proving that the there is no major role for oxidant stress in NASH. Although some data suggest that vitamin E may be helpful, it is not uniformly so.

Long-chain omega-3 fatty acids belong to a family of polyunsaturated fatty acids that are known to have important beneficial effects on metabolism and inflammation. Such effects may confer a benefit in NAFLD. Typically, with a Westernized diet, long-chain omega-6 fatty acid consumption is markedly greater than omega-3 fatty acid consumption. The potential consequences of an alteration in the ratio of omega-6 to omega-3 fatty acid consumption are increased production of proinflammatory arachidonic acid-derived eicosanoids and impaired regulation of hepatic and adipose function, predisposing to NAFLD. If the adipose tissue is inflamed with widespread macrophage infiltration, the production of adipokines may act to exacerbate liver inflammation and NASH. Omega-3 fatty acid treatment may have beneficial effects in regulating hepatic lipid metabolism, adipose tissue function, and inflammation. Recent studies testing the effects of omega-3 fatty acids in NAFLD are showing promise and suggesting that these fatty acids may be useful in the treatment of NAFLD[9].

Some of the animal and human studies have shown that NASH can be caused by events that occur in tandem with fat build-up but are not necessarily caused by it. Instead, in a parallel process in the setting of insulin resistance, the liver needs to metabolize an excess of free fatty acids (FFAs) that are filtered from the bloodstream or are produced by the liver itself. Some of these FFAs are stored as triglycerides that become fat droplets in the liver[10]. Others become toxic intermediates that cause the inflammation and scarring that are associated with NASH. This theory is known as the lipotoxicity model of NASH, and the resultant fat that builds up in the liver, may represent a protective response in the liver’s processing of FFAs. It turns them into inert triglycerides so that they can be stored and metabolized in future. This means that the circulating FFAs generated by adipose tissue or made by the liver from carbohydrates like fructose, are the real offender in NASH[10][11]. Both obesity and dietary contributions to the disease can be explained by this theory, but there are still no hard data in humans to support it. However, the majority of current research is focused on exploring this idea. Another observation made from animal studies was that the trans fats in diet was found to be a greater contributing factor to the development of severe NASH in mice when compared to high-fat & high fructose corn syrup in their diet. This same observation has not yet been made in humans[13]. On contrary another study showed that non-genetically modified mice maintained on a High-Fat- High-Carbohydrate diet (HFHC) diet for 16 weeks in addition to developing obesity, have increased hepatic reactive oxygen species (ROS) and a NASH-like phenotype with significant fibrosis. The mechanism of fibrosis may involve fructose inducing increased ROS associated with CD11b+F4/80+Gr1+ hepatic macrophage aggregation resulting in TGFβ1 signaled collagen deposition and histologically visible hepatic fibrosis[14].

Feutin a protein secreted by liver is increasingly thought to be an important mediator of hepatic insulin resistance. A study was conducted on a group of children with metabolic syndrome and fatty liver including 36 obese and 14 lean children[15]. The study group was subjected to exercise as well as a lifestyle modification. It was noticed that obese children with NAFLD had significantly higher fetuin-A levels than obese children without[16].

The innate immune system also plays a major role in the pathogenesis of NASH. Previously, it was shown that the classical and lectin branches of the complement system are involved in the progression of NAFLD in a significant proportion of patients[17]. NAFLD severity was associated with accumulation of activation products of C3, the central complement component, around steatotic hepatocytes. Several components of the classical and lectin pathways, including C1q, MBL, and C4d, were also found to accumulate in the liver of subjects with NAFLD. However, C3 activation was not accompanied by C1q, MBL, or C4d deposition in all patients, suggesting that the alternative pathway could also be involved in complement activation in NAFLD[18].

Steatotic liver is susceptible to the action of next insults that may exacerbate steatosis and promote NASH. These NASH promoters include: imbalance of production/release of hormones derived from adipose tissue (adipocytokines) with consequent necroinflammation, oxidative stress, activation of specific nuclear receptors activation, and fibrogenesis[19]. In response to the systemic insulin resistance, pancreatic β-cells increase insulin hypersecretion accelerating liver fat accumulation and leading to NAFLD.

Recently, it has been reported that also gut-liver axis may play a crucial role in this complex network of multiple interactions[20]. In fact, it has been suggested that the diet-dependent increase of gut microbiota products may influence intestinal permeability and activate molecular mechanisms of innate immune response, acting as possible inductor of necro-inflammatory lesions and severe fibrosis in NAFLD[21].

Autophagy is crucial for development, differentiation, survival, and homeostasis. Autophagy, or cellular self-digestion, is a catabolic process that targets cell constituents including damaged organelles,
unfolded proteins, and intracellular pathogens to lysosomes for degradation. Important links between the regulation of autophagy and liver complications associated with obesity, non-alcoholic fatty liver disease (NAFLD), have been reported. The spectrum of these hepatic abnormalities extends from isolated steatosis to non-alcoholic steatohepatitis (NASH), steatofibrosis, which sometimes leads to cirrhosis, and hepatocellular carcinoma.

**Biomarkers of NASH**

1. **Markers of Apoptosis**
   Clinical data and animal models suggest that hepatocyte death is the key trigger of liver disease progression, manifested by the subsequent development of inflammation, fibrosis, cirrhosis, and hepatocellular carcinoma. Modes of hepatocellular death differ substantially between liver diseases. Different modes of cell death such as apoptosis, necrosis, and necroptosis trigger specific cell death responses and promote progression of liver disease.

   The disease progression to NASH has been attributed to increased cell death in liver. Apoptosis, or programmed cell death, is a highly organized process that can occur via 2 fundamental pathways: extrinsic mediation by death receptors (such as Fas) or intrinsic mediation by organelles (such as mitochondria). Both pathways can lead to the activation of effector caspases (mainly caspase 3), which cleave different intracellular substrates, including cytokeratin (CK18), which is the major intermediate filament protein in hepatocytes. Caspase-generated CK18 fragment levels can be measured in plasma using the M30 monoclonal antibody enzyme-linked immunosorbent assay (ELISA), and these levels have been found to be significantly higher in NASH patients than simple steatosis (SS) patients. It is the most promising noninvasive test for diagnosing and managing NAFLD. Unfortunately, this test cannot be readily performed because the assay is not yet commercially available, and there is no established CK18 cutoff value for identifying NASH. Another research group found significantly higher levels of plasma soluble Fas (sFas) in NASH patients than in SS or control patients. They developed a prediction model for NASH that included CK18 fragment levels and sFas levels. However, this model must be validated externally and in larger studies before it can be used in clinical practice.

2. **Markers of Oxidative Stress**
   Oxidative stress plays a central role in hepatocyte injury and disease progression from SS to NA34, but precise molecular species has not yet been identified. A study conducted on patients found that they had significantly higher levels of both oxidized low-density lipoprotein and thiobarbituric acid–reacting substances as when compared to controls. On the other hand, another study found significantly lower levels of antioxidant enzyme paroxonase 1 in the NASH cohort. However, there was no correlation between the level of this enzyme and the grade and stage of NAFLD.

   Mass spectrometry was able to detect significantly elevated plasma levels of products of free radical–mediated oxidation of linoleic acid (9- and 13-hydroxy octadecadienoic acid and 9- and 13-oxo-octadecadienoic acid) in NASH patients when compared to patients with SS or normal biopsies.

3. **Markers of Inflammation**
   One of the important factors that may contribute to disease progression to NASH is the chronic inflammatory state that exists in obesity and NAFLD. Nonalcoholic steatohepatitis patients exhibited higher levels of proinflammatory cytokines [tumor necrosis factor-α and interleukin (IL)-6] when compared to SS patients. Unfortunately, we cannot use these cytokines as noninvasive markers for predicting the presence of NASH because the differences have not been significant enough. Many potential biomarkers have been studied with conflicting results. These include cytokines (IL-1β and macrophage inflammatory proteins) and adipokines (resistin, visfatin, and retinol-binding protein 4). Recently, a novel noninvasive marker of NAFLD severity has been identified. It involves measuring the blood neutrophil to lymphocyte (N/L) ratio. This ratio was found to be higher in patients with NASH than those with SS. The blood N/L ratio was shown to correlate with the main histologic features of NAFLD, including inflammation and fibrosis. Recently, Knowlley and coauthors demonstrated that ferritin levels more than 1.5 times the upper limit of normal were associated with the diagnosis of NASH and advanced fibrosis in a large cohort of biopsy-confirmed NAFLD patients who were enrolled in the NASH Clinical Research Network.

4. **Markers of Fibrosis**
   Differentiation of NASH from bland steatosis in adult patients with NAFLD requires the combination of clinical, biochemical and/or extracellular matrix protein-associated serum markers. An enhanced liver fibrosis (ELF) score, is calculated from an algorithm that incorporates a panel of hyaluronic acid (HA), amino-terminal propeptide of type III collagen (PIIINP), and tissue inhibitor of metalloproteinase 1 (TIMP-1). The ELF score has a reasonable predictive capability in adult NASH.

**Liver Pathology in NASH**

A major & challenging problem in diagnosing of Pediatric NASH is the fact that the histological findings that are commonly found in Adult NASH are less commonly seen in children with definitive NASH. These histological findings include ballooning degeneration, classic zone-3 fibrosis and parenchymal inflammation. To better define pediatric NASH, a study was conducted and 100 consecutive liver biopsies of children with NASH were analyzed. Simple steatosis was present in 16% of the patients, 8% had advanced fibrosis, 3% had cirrhosis and the rest had NASH. Two distinct pathologic subtypes were present in these children with NASH. This proves that histopathological findings of NASH in children often differ from that found in adults. Two-subtypes were proposed. Type 1 NASH (resembling an adult-type pattern) was seen in only 17% of children and was characterized by steatosis with ballooning degeneration and lobular inflammation, with or without perisinusoidal fibrosis, and without portal inflammation. Type 2 NASH was found in 51% of children and it was characterized by macrovesicular hepatocellular steatosis with portal inflammation, with or without portal fibrosis, and no or minimal ballooning degeneration. The remaining patients (32%) had a combination of features common to both the type 1 and 2 NASH pattern.

**Histologic Scoring System in NASH**

Pediatric NAFLD interpretation has a lesser degree of inter-observer agreement than adult interpretations of NAFLD. Therefore, standard nomenclature and a uniform histologic scoring system for reading biopsy specimens of patients with suspected NASH is important.

The proposed histological scoring system in patients with NASH...
is mainly based upon adult patients with NASH and NAFLD. Until further refinements in the pediatric NAFLD scoring system are developed, we suggest that investigators utilize the NAFLD scoring system designed and validated by the pathology committee of the NASH-Clinical Research Network (NASH-CRN)\[43\]. The scoring system is composed of 14 histologic features; four of the key features are evaluated semi-quantitatively including lobular inflammation (0-2), hepatocellular ballooning (0-2), steatosis (0-3), and fibrosis (0-4). The other nine features, which include acidophil bodies, microgranulomas, Mallory hyaline, lipogranulomas, megamitochondria, pigmented macrophages, microvesicular steatosis, portal inflammation, and glycogenated nuclei, are qualitatively assessed as present or absent. This system also provides a NAFLD Activity Score (NAS) that can be utilized in clinical trials of NASH. NAS (range: 0-8) is the sum of ballooning degeneration, lobular inflammation, and steatosis scores. Diagnosis of NASH correlates well with a NAS score of 5 or more, while patients with NAS score of less than 3 are generally classified as "non-NASH". NAS scores of 3 or 4 are considered borderline for a diagnosis of NASH and these cases may benefit from assessment of the entire biopsy specimen, utilizing other features of NASH histology. NAS is not a measure of rapidity of disease progression, and it is unclear if patients with NASH who have a higher NAS score have a worse prognosis\[43\]. As emphasized by Sanjal and colleagues, the presence of steatohepatitis cannot be inferred from the NAS and requires an overall assessment of the presence and distribution of the individual histologic findings. The NAS should not be considered to be either "synonymous" with, or actually a replacement for, a microscopic diagnosis that is based on overall pattern of injury (i.e. steatosis, hepatocyte ballooning, and inflammation), as well as the presence of additional lesions (i.e. zonality of lesions, portal inflammation, and fibrosis)\[44\]. Brunt et al showed that the use of an NAS cutoff more or equal to 5 versus less or equal to 4 as a substitute for the histological diagnosis of steatohepatitis (definite steatohepatitis versus borderline/ not steatohepatitis) yielded a sensitivity of 75%(95% CI: 72-78), specificity of 83%(80-85), percent agreement of 78.4% (75.6-81.0) and Cohen’s kappa statistic of 57% (51-62). Taken together, these measures indicate a substantial loss in information if the NAS were used as a surrogate for the diagnosis of NASH\[45\].

Therefore, we would like to emphasize that NAS should not be taken as an absolute severity scale for NASH nor may it be used as a diagnostic tool for NASH. It should rather be used as an objective scoring system for reporting and assessing treatment response in NASH studies in a standardized manner.

**MANAGEMENT**

The overall goal of therapy is to reduce long-term cardiovascular and liver morbidity & mortality and to improve the individual's quality of life. Up to date there is no approved pharmacologic therapy for NAFLD or NASH. Currently, the contemporary management standards are primarily based upon the presence of associated risk factors in an NAFLD patient.

**DIET AND EXERCISE**

We must work at targeting pediatric obesity because the commonest findings in NAFLD patients are obesity and overweight. This should help in reducing the burden of pediatric NAFLD. Studies have shown that there was a marked improvement in serum ALT and liver histology after weight loss in adults with NAFLD\[46\]. The efficacy and degree of weight loss that is required needed to induce histologic improvement in pediatric NAFLD is still unknown. Studies of adult NAFLD proved that a weight loss of more than 5 % correlated with significant improvement in liver histology. A pediatric open label study showed that a mean weight loss of approximately 5 kg (baseline weight: 61 kg; age range: 5-19 years) resulted in improvement in serum ALT and AST in most children with NAFLD\[47\]. However, randomized-controlled trials (RCT) are needed to assess the efficacy of weight loss in relation to the histologic improvement in pediatric NASH.

No information exists on recommending any type of diet. A low-carbohydrate diet has been shown to lead to reduction in serum ALT and hepatic steatosis\[49\]. However, we cannot rely on serum ALT alone as a marker for histologic severity. Weight loss in obese adolescents and adults can be effectively achieved by following a low-glycemic index diet as compared to a low fat diet\[50\], but has yet to be tested in children with NASH.

It is highly recommended that all overweight pediatric NAFLD patients must consult and follow-up with a qualified dietitian in order to assess the quality of their diet and daily caloric intake. They should follow the diet strategies adopted by American Heart Association (for children aged >2 years). The importance of regular aerobic exercise progressing in difficulty as fitness allows must be stressed on at all times\[51\]. Strict diet control and exercise compliance by NAFLD patients can be aided through enlisting other willing family members to adopt the same lifestyle. This will help the patients to achieve their goals effectively. Future studies utilizing these interventions and behavioral modifications should be tested in pediatric obesity and NAFLD to develop more evidence-based recommendations\[52\].

**Vitamin E/vitamin C**

Many studies involving vitamin E have been conducted\[52-54\], and they have shown promising reduction of aminotransferases. A placebo controlled trial noticed an improvement in hepatic inflammation and fibrosis when a combination of vitamin E and C was used\[54\]. However, no statistical significant differences were noted between the cases and placebo group. In an open label study, vitamin E was found to be inferior to metformin and/or weight loss in improving aminotransferases\[56\].

**Ursodiol**

The precise mechanistic role of ursodiol in the treatment of NASH is unknown. It might exert its effect (cytoprotective effect) by reducing bile-salt mediated mitochondrial injury within hepatocytes. However, it failed to show any benefit in RCT in adults with NASH and children with NAFLD. An RCT conducted in obese children with elevated serum ALT reported that ursodiol (10-12.5 mg/kg orally per day) was ineffective in improving serum ALT or steatosis\[57\]. Another RCT in NASH patients showed that the combination of ursodiol with vitamin E may improve serum ALT and steatosis\[58\].

**Metformin**

The only insulin-sensitizing agent evaluated for the treatment of NAFLD in children is metformin. It improves insulin sensitivity via activation of LKB1, a tumor suppressor gene, which phosphorylates and activates AMP-activated protein kinase and thereby decreases gluconeogenesis in the liver. It is suggested that metformin tends to improve NASH in adults by inducing weight loss\[59\].

Diabetic children can be safely and effectively treated with metformin. This has encouraged researchers to try the drug in children with NASH. A pilot study administered metformin in
pediatric NASH at a dose of 500 mg twice daily for a period of six months. This resulted in improvement in serum ALT and hepatic steatosis as assessed with magnetic resonance spectroscopy (MRS) [60].

Thiazolidinediones
Thiazolidinediones such as rosiglitazone and pioglitazone have been evaluated in adult patients with NASH and appear promising. Two RCTs showed that pioglitazone was effective in improving NASH histology but a rosiglitazone study did not show any benefit. Rosiglitazone is unlikely to be tested in children because of the serious concerns regarding its cardiovascular safety profile. A major side-effect of this group of medications that might limit its long term use is weight gain. This is due to increase in adiposity that appears to persist even after cessation of medication[61]. In the largest controlled trial to date with pioglitazone, Belfort et al compared diet plus pioglitazone to diet plus placebo in 55 patients. The pioglitazone group showed significant improvement in ALT (by 58%), hepatic fat content (by 54%) and insulin sensitivity (by 48%). There was significant histologic improvement in steatosis, ballooning necrosis and inflammation but not fibrosis[62]. Well-designed studies in pediatric NASH are necessary before considering pioglitazone in clinical practice.

Orlistat
In obese patients with NASH, liver fibrosis and inflammation improved after therapy with orlistat. Orlistat is a lipase inhibitor that promotes weight loss by reduction of fat absorption. A study was conducted to evaluate the efficacy of orlistat, when given for 6 months to patients with obesity and biopsy confirmed NASH. It was found that orlistat therapy and dietary counselling were associated with significant decreases in body weight, haemoglobin A1c, alanine aminotransferase (ALT) and aspartate aminotransferase (AST). A 10% or greater reduction in weight improved steatosis and fibrosis as well as haemoglobin A1c levels in the majority of patients treated for 6 months. Controlled trials of longer duration are warranted to assess for histopathologic improvement as well as cost-efficacy in comparison to diet and exercise alone[63].

Statins
Statins may reduce hepatic fat content in patients with hyperlipidaemia and NASH. To date, atorvastatin, pravastatin, and rosuvastatin have been studied. An important point about these studies is that the statin doses were not equipotent nor comparable in their lipid lowering effect[64].

Docosahexaenoic acid
Docosahexaenoic acid (DHA) is the major dietary N-3 LC-PUFA. The short-term (6 months) and long-term (up to 24 months) effects after 6, 12, 18 and 24 months of treatment with different concentrations of DHA (250 mg/day and 500 mg/ day) combined with diet and exercise was studied.

In these studies, algae DHA supplementation improved liver steatosis in children with NAFLD and was able to reduce the levels of serum ALT and triglycerides[65,66]. DHA exerts a potent anti-inflammatory activity through the G protein-coupled receptor GPR120[67]; dietary DHA suppresses hepatic markers of oxidative stress, inflammation and fibrosis[68]. DHA could modulate hepatic progenitor cell activation, hepatocyte survival and macrophage polarization through the interaction with GPR120 and NF-kB repression[69].

Pentoxifylline
Pentoxifylline is a methylxanthine derivative with potent hemorrhheologic properties. Pentoxifylline, as a nonspecific phosphodiesterase inhibitor, results in a variety of physiological changes at the cellular level, increases levels of cyclic AMP and decreases tumor necrosis factor (TNF)-α gene transcription[70], affecting multiple steps in the cytokine/chemokine pathway. Increased serum TNF-α has been reported in humans and animal models of NAFLD[71] and may be important in treating NAFLD. Use of pentoxifylline can reduce body weight, serum ALT, AST, glucose and TNF-α. With regard to histological changes, pentoxifylline could only reduce the NAFLD activity score and improved lobular inflammation. Thus, pentoxifylline may represent a new method for treating or preventing NAFLD[72].

Pro-biotics/pre-biotics
Probiotics may reduce hepatic injury in animal models where intestinal derived bacterial endotoxin sensitizes fatty livers to the effects of TNF-α. A 3-month treatment period of a commercially available probiotic, VSL #3, given to 22 patients with NAFLD did improve ALT levels and markers of lipid peroxidation. Histology was not evaluated in this trial[73].

Bariatric surgeries
Current evidence suggests that bariatric surgery can decrease the grade of steatosis, hepatic inflammation and fibrosis in non-alcoholic steatohepatitis. Uncomplicated NAFLD is not an indication for bariatric surgery. Roux-en-Y gastric bypass is considered a safe and effective option for extremely obese adolescents, as long as appropriate long-term follow-up is provided. Laparoscopic adjustable gastric banding has not been approved by the FDA for use in adolescents and therefore should be considered investigational. Finally, sleeve gastrectomy and other types of weight loss surgery that have grown increasingly common in adults, still need to be considered investigational. Temporary devices may be increasingly being used in pediatrics, however future studies, including a long-term risk analysis of patients who undergo surgery, are much needed to clarify the exact indications for bariatric surgery in adolescents[74].

CONCLUSION
NASH is a complex metabolic disease resulting from a wide variety of insults. Improved understanding of pathogenesis through genetic and familial studies is a priority. Liver biopsy is the gold standard and currently the only way to diagnose NASH. Therefore, we need noninvasive biomarkers for diagnosing patients with steatosis who are at highest risk of disease progression. Large population-based epidemiologic studies are needed to understand the true impact of NASH on long-term morbidity and mortality and its associations with cardiometabolic- cancer risk factors. Lastly, we need effective and safe treatments for NASH that are practical, cost-effective and readily transferable to the community at large.

CONFLICT OF INTERESTS
There are no conflicts of interest with regard to the present study.

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