Diet and Exercise in the Treatment of Fatty Liver

Guest Editors: Faidon Magkos, Jean-Marc Lavoie, Konstantinos Kantartzis, and Amalia Gastaldelli
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In recent years, we came to realize that obesity, broadly defined as increased body mass index or increased total body fat, is not necessarily associated with metabolic dysfunction and greater risk for cardiometabolic disease. In fact, there are several obese persons who are “metabolically healthy,” as there are nonobese persons who are “metabolically abnormal.” Although the reason(s) underlying this phenomenon are still not entirely clear, a number of studies conducted over the past several years indicate that the anatomical location of excess fat is more important than total body adiposity in determining metabolic outcomes.

Ectopic fat accumulation, particularly in the liver, is frequently observed in obese persons and is strongly associated with metabolic dysfunction, including multiorgan insulin resistance and dyslipidemia. Intrahepatic fat, possibly more than visceral or intramyocellular fat, may thus be a prominent factor modifying the metabolic risk associated with increasing whole-body adiposity. However, cause-and-effect relationships have not yet been established, and it is also possible that intrahepatic triglyceride content is not a determinant but merely a marker of metabolic health.

Understanding the regulation of fat accumulation in the liver will thus have important implications in both research and clinical practice. Little is known regarding the specific effects of lifestyle factors such as diet and exercise in regulating the accumulation of fat in the liver and its depletion thereof. In this special issue, we have invited a few papers in an attempt to partly fill this gap in our knowledge.

In the first paper of this issue, “Putative factors that may modulate the effect of exercise on liver fat: insights from animal studies,” several studies in animals are reviewed in order to highlight putative factors that may modulate the effect of exercise on liver fat. This includes the fat content of the diet (exercise appears to be more effective under high-fat feeding), the role of concurrent exercise-induced loss of body weight or visceral fat, sex (males versus females), prandial status (fasted versus fed), and the duration of training, as well as the time elapsed from the last bout of exercise. The potential importance of these factors in modifying the exercise-induced changes in liver fat has not yet been formally tested in man, thereby providing a wide array of opportunities for future research.

The second paper of this issue, “Nafld, estrogens, and physical exercise: the animal model,” focuses on the effects of exercise on liver fat in relation to estrogen availability. Estrogen deficiency, such as that occurring naturally after menopause in women, is strongly associated with fatty liver in animals. Exercise training exerts an estrogen-like effect on the expression of genes involved in hepatic lipid metabolism and is a powerful means for preventing liver fat accumulation in estrogen-deficient animals. The third paper of this special issue, “Dietary conjugated linoleic acid and hepatic steatosis: species specific effects on liver and adipose lipid metabolism and gene expression,” reviews the effects of dietary conjugated linoleic acid on liver fat content and hepatic and adipose tissue fatty acid metabolism in animals. Conjugated linoleic acids, particularly the trans-10, cis-12, lead to hepatic steatosis owing to increased de novo lipogenesis and increased hepatic fatty acid uptake, at rates far exceeding the rates of disposal of intrahepatic fatty acids towards oxidation, esterification, and triglyceride export.
The fourth paper of this issue, “Effects of exercise training on molecular markers of lipogenesis and lipid partitioning in fructose-induced liver fat accumulation,” examines the effects of exercise training on liver fat in starved and subsequently fructose-refed animals. Fructose, a simple sugar, is a potent dietary trigger for liver fat accretion. Exercise training in this model is not able to reverse the fructose-induced changes in lipogenic enzymes and does not reduce intrahepatic fat content. Thus, contrary to the large body of evidence demonstrating that exercise is effective in alleviating hepatic steatosis induced by high-fat feeding, exercise is not able to reverse the changes induced by fructose feeding. The final paper of this special issue, “Exercise and omega-3 polyunsaturated fatty acid supplementation for the treatment of hepatic steatosis in hyperphagic OLETF rats,” evaluates the effects of exercise on a hyperphagic model of obesity, with or without concurrent omega-3 polyunsaturated fatty acid supplementation. Exercise training in this animal model alleviates hepatic steatosis even under low-fat feeding conditions, predominantly by increasing hepatic fatty acid oxidation, whereas supplementation with omega-3 fatty acids slightly increases liver-fat content and attenuates the liver-fat-depleting effect of exercise. It is noteworthy that omega-3 fatty acid supplementation in this study accounted for only 3% of total dietary energy, whereas in several previous studies showing that omega-3 fatty acids reduce liver fat the supplement was administered at much greater doses.

Research presented and reviewed in this special issue not only highlights the independent effects of exercise and diet on liver fat accumulation but also, more importantly, raises the intriguing possibility of interactive effects between exercise and diet on the mechanisms regulating liver fat accretion and depletion. It seems that several dietary factors are able to either augment or attenuate the intrahepatic triglyceride-depleting effect of exercise.

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Putative Factors That May Modulate the Effect of Exercise on Liver Fat: Insights from Animal Studies

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An increase in intrahepatic triglyceride (IHTG) content is the hallmark of nonalcoholic fatty liver disease (NAFLD) and is strongly associated with insulin resistance and dyslipidemia. Although regular aerobic exercise improves metabolic function, its role in regulating fat accumulation in the liver is incompletely understood, and human data are scarce. Results from exercise training studies in animals highlight a number of potential factors that could possibly mediate the effect of exercise on liver fat, but none of them has been formally tested in man. The effect of exercise on IHTG content strongly depends on the background diet, so that exercise is more effective in reducing IHTG under conditions that favor liver fat accretion (e.g., when animals are fed high-fat diets). Concurrent loss of body weight or visceral fat does not appear to mediate the effect of exercise on IHTG, whereas sex (males versus females), prandial status (fasted versus fed), and duration of training, as well as the time elapsed from the last bout of exercise could all be affecting the observed exercise-induced changes in IHTG content. The potential importance of these factors remains obscure, thus providing a wide array of opportunities for future research on the effects of exercise (and diet) on liver fat accumulation.

1. Introduction

Excessive accumulation of fat in the liver, that is, intrahepatic triglyceride (IHTG), is associated with increased prevalence rates of and risk for dyslipidemia, diabetes, and cardiovascular disease [1–3]. Data from epidemiological as well as metabolic studies indicate that increased IHTG content is accompanied by insulin resistance and dysregulation of lipid metabolism [4–6]. Exercise is known to improve metabolic function [7, 8]; however its effects on IHTG remain elusive [9, 10]. Data from studies in humans are scarce and not entirely consistent [11]. In this paper, the results from a number of animal studies are briefly reviewed in an attempt to highlight putative factors that may modulate the effect of exercise on IHTG content.

2. Exercise Training in Animals

Many studies have evaluated the effect of aerobic exercise training on IHTG content in rodents; their design varies in terms of sex, strain, background diet, training duration, the prandial status, and the time of assessment after the last bout of exercise (Table 1) [12–37]. Results are largely heterogeneous, but a crude analysis of the data suggests that endurance training decreases IHTG (median: −16%, range: −92% to +97%, n = 50 studies; Table 1). Most frequently [14, 16, 18, 20, 26, 31, 35] but not always [15, 17, 34, 37], exercise has been shown to be more effective in reducing liver fat or attenuating its accretion in animals fed high-fat rather than standard, low-fat diets (median decrease: 25% and 14%, resp., Figure 1(a)). This is consistent with data from human studies, in which exercise training appears to be more potent in reducing IHTG in subjects with increased baseline IHTG, for example, subjects with NAFLD, type II diabetes, or the elderly [11].

The reasons why exercise is more effective in reducing IHTG on high-fat than low-fat diets are not entirely clear but are likely related to the hepatosteatotic effect of high-fat feeding. Fat is mainly stored as microvesicles (<1 μm²) within
Table 1: Effect of aerobic exercise training on liver fat in animals.

| Study                  | Animals                                  | Diet                                      | Intervention                      | Duration | Withdrawal before measurements | Effect of training (EX versus respective SED group on the same diet) |
|------------------------|------------------------------------------|-------------------------------------------|-----------------------------------|----------|---------------------------------|---------------------------------------------------------------------|
| Ahrens et al., 1972    | Male Wistar rats; young and mature       | HF (ad libitum or pair-fed) with two different carbohydrate sources | SED or EX (1/d, running, 30 min at \sim 13 m/min and 0% incline) | 8 wk     | 12 h                            | Young: –15%<br>Mature: –35%                                         |
| Barakat et al., 1987   | Female rats; control and alloxan-diabetic | SC? (ad libitum)                          | SED or EX (1/d, running, 2 h at 20 m/min and 0% incline) | 7 d      | 0 h                             | Control: –14%<br>(NS)<br>Diabetic: –2% (NS)                          |
| Cha et al., 1999       | Male Sprague-Dawley rats                 | SC or HF (ad libitum)                     | SED or EX (1/d, running, 1.5 h at 150 m/min and 1% incline) | 1 mo     | ?                               | SC: /?<br>HF: /?<br>SC: –24%<br>HF: –40%                            |
| Chapados et al., 2009  | Female Sprague-Dawley rats               | SC or HF (ad libitum)                     | SED or EX (5/wk, running, progressive until 1 h at 26 m/min and 10% incline for the last 4 wk) | 8 wk     | 12 h                            | SC: /?<br>HF: /?<br>SC: –16% (NS)<br>HF: –22% (NS)                  |
| Charbonneau et al., 2005 | Female Sprague-Dawley rats               | SC or HF (ad libitum)                     | SED or EX (6/wk, running, progressive until 1 h at 26 m/min and 10% incline for the last 3 wk) | 6 wk (+2 wk diet lead-in) | 2-3 h 48 h | SC: /?<br>HF: /?<br>SC: 0% (NS)<br>HF: –14%                            |
| Fukuda et al., 1991    | Male Wistar rats                         | SC, HF or HChol (ad libitum)              | SED or EX (voluntary running)      | 4 wk     | 3 h                             | SC: /?<br>HF: /?<br>HChol: /?                                          |
| Gauthier et al., 2003  | Female Sprague-Dawley rats               | SC or HF (ad libitum)                     | SED or EX (5/wk, running, progressive until 1 h at 26 m/min and 10% incline for the last 4 wk) | 8 wk     | 2 h                             | SC: /?<br>HF: /?<br>SC: +16% (NS)<br>HF: –29%                        |
| Gauthier et al., 2004  | Female Sprague-Dawley rats               | HF (ad libitum)                           | SED or EX (5/wk, running, progressive until 1 h at 26 m/min and 10% incline for the last 4 wk) | 8 wk (+8 wk diet lead-in) | 2 h 48 h | /?<br>−16% (NS)                                                          |
| Gollisch et al., 2009  | Female Sprague-Dawley rats               | SC or HF (ad libitum)                     | SED or EX (voluntary running)      | 4 wk     | 10 h                            | SC: /?<br>HF: /?<br>SC: –16% (NS)<br>HF: –31%                        |
Table 1: Continued.

| Study                              | Animals                                                                 | Diet                        | Intervention Exercise | Duration | Withdrawal before measurements | Effect of training (EX versus respective SED group on the same diet) |
|------------------------------------|-------------------------------------------------------------------------|-----------------------------|-----------------------|----------|--------------------------------|---------------------------------------------------------------------|
| Ho et al., 2010 [21]               | Female Sprague-Dawley rats; sham-operated or OVX with and without E2  | SC (ad libitum)            | SED or EX (5/wk, running, 1 h at 18 m/min and 0% incline) | 12 wk    | 24 h                           | Sham: ↔/↔ OVX: ↓/↓ OVX+E2: ↔/↔                                         |
| Karanth and Jeevaratnam, 2009 [22] | Male Wistar rats                                                        | HF rich in SFA or MUFA (ad libitum) with carnitine or not | SED or EX (6/wk, swimming, 1 h) | 6 mo     | Overnight 20 h ↔/? (both diets) | SEA: −35% MUFA: −25% (average with or without carnitine)            |
| Lessard et al., 2007 [23]          | Male Sprague-Dawley rats                                               | HF (ad libitum)             | SED or EX (5/wk, running, progressive until 1 h at 32 m/min and 15% incline for the last 3 wk) | 4 wk (+4 wk diet lead-in) | 8–12 h 36–48 h | // | −41% |
| Lira et al., 2008 [24]             | Male Wistar rats; control and tumor-bearing                             | SC (ad libitum)            | SED or EX (5/wk, running, progressive until 1 h at 20 m/min and 0% incline for the last 2 wk) | 8 wk     | 24 h                           | /? (both groups) Control: −30% Tumor: −92%                          |
| Morifuji et al., 2006 [25]         | Male Sprague-Dawley rats                                               | SC with casein or soya as protein source (ad libitum) | SED or EX (6/wk, swimming, 2 h) | 2 wk     | Nonfasting 24 h | // (both groups) | Casein: −21% Soya: −24% |
| Narayan et al., 1975 [26]          | Male Holtzman rats                                                      | SC or HF (ad libitum)       | SED or EX (5/wk, running, progressive until 80–85 min at 23 m/min and 8.5% incline for the last 3 wk) | 6 wk     | Nonfasting 24 h | /? | SC: +81% (NS) HF: −51% |
| Petridou et al., 2005 [27]         | Male Wistar rats                                                        | SC (ad libitum)             | SED or EX (voluntary running) | 8 wk     | 6 h 12 h | /? | −12% (NS) |
| Pighon et al., 2010 [28]           | Female Sprague-Dawley rats                                             | SC (ad libitum)             | SED or EX (5/wk, running, progressive until 1 h min at 26 m/min and 10% incline for the last 4 wk) | 6 wk     | 3 h 48 h | ↔/↓ | +1% (NS) |
| Pighon et al., 2010 [28]           | Female Sprague-Dawley rats; sham-operated or OVX with and without E2  | SC (ad libitum)             | SED or EX (5/wk, running, progressive until 1 h min at 26 m/min and 10% incline for the last 3 wk) | 5 wk     | 3 h 48 h | // (all groups) | Sham: −1% (NS) OVX: −26% OVX+E2: −5% (NS) |
| Study                                    | Animals                                      | Diet                          | Intervention Exercise | Duration | Withdrawal before measurements | Effect of training (EX versus respective SED group on the same diet) |
|-----------------------------------------|----------------------------------------------|-------------------------------|-----------------------|----------|---------------------------------|---------------------------------------------------------------|
| Rector et al., 2008 [29]               | Male OLETF rats (obese and diabetic)         | SC (ad libitum)              | SED or EX (voluntary running) | 16 wk    | 5 h                             | ↓/↓− 45%                                                      |
| Rothfeld et al., 1977 [30]             | Male Sprague-Dawley rats (pair-fed)          | HF (pair-fed)                 | SED or EX (voluntary running) | 3 wk     | ?                              | ↓/↓− 14%                                                      |
| Straczkowski et al., 2001 [31]         | Male Wistar rats                             | SC for wk 0–3 and SC or HF for wk 4–6 (pair-fed) | SED or EX (6/wk, running, 3 h at 20 m/min and 10% incline) | 6 wk     | ?/8 h (both diets)              | SC: +97% HF: −9% (NS)                                        |
| Terao et al., 1987 [32]                | Male Wistar rats                             | HChol (ad libitum)           | SED or EX (5/wk, running, progressive until 1 h at 20 m/min and 0% incline for the last 2 wk) | 5 wk (+5 wk SC diet lead-in) | ?                    | ?/− 16% (NS)                                                  |
| Tsutsumi et al., 2001 [33]             | Male Sprague-Dawley old rats                 | SC (ad libitum)              | SED or EX (1/d, running, 30 min at 15 m/min and 10% incline) | 3 mo     | ~12 h                           | −41%                                                          |
| Vieira et al., 2009 [34]               | Male C57BL/6, HF diet-induced obese mice     | SC or HF (ad libitum)        | SED or EX (5/wk, running, 40 min at 12 m/min and 12% incline) | 6 wk     | 12 h                            | SC: −/−/− SC: −49% (NS) HF: −11% (NS)                         |
| Vieira et al., 2009 [34]               | Male C57BL/6, HF diet-induced obese mice     | SC or HF (ad libitum)        | SED or EX (5/wk, running, 40 min at 12 m/min and 12% incline) | 12 wk    | 12 h                            | SC: −/−/− SC: −72% HF: −48%                                  |
| Vieira et al., 2009 [35]               | Male Balb/cByl mice (with defective fatty acid oxidation) | SC or HF (ad libitum)        | SED or EX (5/wk, running, 1 h at 12 m/min and 5% incline) | 12 wk    | 12 h                            | −SC: −/−/− SC: −5% (NS) HF: −40% (P = 0.09)                  |
| Yasari et al., 2006 [36]               | Female Sprague-Dawley rats                   | SC (ad libitum)              | SED or EX (5/wk, running, progressive until 1 h at 26 m/min and 10% incline for the last 4 wk) | 8 wk     | 3 h                             | −/−/−/− SC: −9% (NS)                                         |
| Yasari et al., 2010 [37]               | Female Sprague-Dawley rats                   | SC for wk 0–6 and SC or HF for wk 7–8 (ad libitum) | SED or EX (5/wk, running, progressive until 1 h at 26 m/min and 10% incline for the last 4 wk) | 8 wk     | 3–48 h                          | SC: −/−/−/− SC: −13% (NS) HF: +7% (NS)                       |

All changes shown are statistically significant versus control group (SED), unless indicated otherwise (- is unchanged; ↓ is reduced; NS is not significant; ? is unknown).

BW: body weight; E2: estradiol; EX: exercised; HChol: high fat and cholesterol; HF: high fat; MUFAs: monounsaturated fatty acids; O VX: ovariectomized; SC: standard chow (low fat); SED: sedentary; SFA: saturated fatty acids; VAT: visceral adipose tissue (mesenteric, retroperitoneal, and/or epididymal fat pads).
Figure 1: Factors that may affect changes in liver fat in response to exercise training in animals. Exercise-induced changes in intrahepatic triglyceride content (Δ-IHTG) are shown for: (a) animals fed high fat or standard chow (low fat) diets; (b) animals that experienced weight loss (or attenuated weight gain) or not; (c) male or female animals; (d) fasted or fed animals; (e) animals trained for longer or shorter periods of time; (f) animals examined within one day from the last bout of exercise or later during recovery. Box plots have been constructed using average changes in liver fat (% difference relative to sedentary controls) for each group of animals in the studies depicted in Table 1, and illustrate median, first, and third quartiles, minimum and maximum values, as well as potential positive and negative outliers.
well as with recent observations in humans [44]. Studies immediately after exercise, in female rats because exercise (30–60 min) did not affect changes in total body fat [40] or even visceral adipose tissue reductions in IHTG content can occur in the absence of other conditions that favor the development of fatty liver, such as overfeeding [34], ovariectomy [28], ethanol ingestion [39], or tumor-bearing [24]. Apart from the fat content of the background diet, the type of dietary carbohydrate [12], protein [25], and fat (i.e., saturated or unsaturated fatty acids) [22], as well as the feeding pattern (ad libitum or paired) [12] do not appear to affect, at least not in a major way, the exercise-induced change in IHTG content.

The collective of available data in animals highlights a number of other putative factors that may modulate the effect of exercise on liver fat; however, none of these factors has been formally tested using rigorous experimental designs. Concurrent weight loss or attenuated weight gain is not likely critical for the exercise-induced depletion of IHTG to manifest, albeit they may lead to greater reductions in liver fat when compared to no weight loss or similar weight gain (median decrease: 27.5% and 14%, resp., Figure 1(b)). However, just like in humans [11], loss of visceral adipose tissue mass with exercise training is not necessarily coupled with a corresponding decrease in liver fat [16, 18, 28, 37]. Likewise, human studies have shown that exercise-induced reductions in IHTG content can occur in the absence of changes in total body fat [40] or even visceral adipose tissue [41].

Exercise may be more effective in reducing IHTG content in males than in females (median decrease: 25% and 14%, resp., Figure 1(c)), in fasted (≥6 h) than in fed animals (median decrease: 31% and 11%, resp., Figure 1(d)), and after longer (≥8 wk) than shorter interventions [34] (median decrease: 24% and 14%, resp.; Figure 1(e)). The time elapsed from the last bout of exercise (≤24 h or ≥36 h) may also mediate the observed changes in IHTG (median decrease: 24% and 13.5%, resp., Figure 1(f)), suggesting that even acute exercise could affect liver fat. However, relevant information is scarce and inconclusive. A single bout of aerobic exercise (30–60 min) did not affect IHTG content, measured immediately after exercise, in female rats [42] but caused a ~30% decrease in male rats [43] of the same strain, under both standard and high-fat feeding conditions. This is in line with data from exercise training studies in animals raising the possibility that males may be more sensitive to the IHTG-reducing effect of exercise than females (Figure 1(c)), as well as with recent observations in humans [44]. Studies in which male rats were exercised until exhaustion provide conflicting results, some observed a mild [45] or marked [46, 47] increase in hepatic steatosis whereas others found a decrease of 30–60% [48] at the end of exercise.

3. Detraining after Regular Exercise

If regular exercise reduces liver fat, cessation of exercise should lead to an increase in IHTG content. Only a few animal studies have evaluated the effect of detraining on liver fat accumulation, and all have demonstrated that cessation of regular exercise (after 6–16 weeks of training) for a short (2–3 days) or a long (6 weeks) period of time is not associated with any significant changes in IHTG content compared with the trained state (i.e., before discontinuation of exercise) when animals are fed a standard low-fat diet [28, 36, 49, 50]. Furthermore, detraining for 2–7 days does not alter the total number of hepatocyte lipid vacuoles and their size, even though it does activate precursors and processes in the liver known to initiate steatosis (e.g., decreased mitochondrial oxidative capacity, increased expression of de novo lipogenesis proteins, and increased malonyl CoA levels) [49]. Interpretation of detraining data is not straightforward, though. It is possible that this lack of an effect of detraining relates to the lesser potency of exercise in reducing liver fat content in animals fed standard low-fat diets (Figure 1), so that changes after detraining are similarly less pronounced. For instance, two [28, 36] out of three studies that reported no effect of detraining on liver fat also failed to observe a training-induced decrease in IHTG content, suggesting that training and detraining have no effect on liver fat accumulation under low-fat feeding conditions. Whereas one study [49] did observe a training-induced decrease in liver fat in rats fed a low-fat diet but found no changes after detraining, implying that the IHTG-depleting effect of regular exercise is long-lived and is not readily reversed by detraining. Still, compared with sedentary, never-exercised counterparts, detrained animals appear to be relatively protected from mild hepatic steatosis induced by 2 weeks of high-fat feeding [36], but not from the development of frank fatty liver 6 weeks after ovariectomy [28], even though cessation of exercise training in ovariectomized rats resulted in a nearly 40% increase in IHTG content compared with ovariectomized rats who did not stop exercising [28]. It is thus possible that the relevant molecular and biochemical adaptations to exercise are readily reversed when the exercise routine is interrupted (<1 week), however, changes in IHTG manifest later in time and only when strong triggering factors for liver fat accretion coexist, such as high-fat feeding or ovariectomy. Support for this notion is provided by an earlier study, where detraining resulted in a striking increase in IHTG content only when animals were subjected to starvation and refeeding [50].

4. Conclusion

The effect of exercise on IHTG content has recently attracted much scientific interest in light of the apparent detrimental metabolic effects of excessive liver fat accumulation. Although the results from a few studies in human subjects are promising, as exercise appears to reduce IHTG [11], the importance of the factors highlighted herein on the basis of studies in animals has never been evaluated in man. Future studies should at least control for—in order to avoid
confounding—or directly investigate the role of these factors in affecting the exercise-induced changes in liver fat content.

**Conflict of Interests**

The author declares that there is no conflict of interests.

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Review Article
NAFLD, Estrogens, and Physical Exercise: The Animal Model

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One segment of the population that is particularly inclined to liver fat accumulation is postmenopausal women. Although nonalcoholic hepatic steatosis is more common in men than in women, after menopause there is a reversal in gender distribution. At the present time, weight loss and exercise are regarded as first line treatments for NAFLD in postmenopausal women, as it is the case for the management of metabolic syndrome. In recent years, there has been substantial evidence coming mostly from the use of the animal model, that indeed estrogens withdrawal is associated with modifications of molecular markers favouring the activity of metabolic pathways ultimately leading to liver fat accumulation. In addition, the use of the animal model has provided physiological and molecular evidence that exercise training provides estrogens-like protective effects on liver fat accumulation and its consequences. The purpose of the present paper is to present information relative to the development of a state of NAFLD resulting from the absence of estrogens and the role of exercise training, emphasizing on the contribution of the animal model on these issues.

1. Introduction

Liver is particularly vulnerable to ectopic fat accumulation that results in NAFLD characterized by hepatic lipid accumulation (5 to 10% per weight) in the absence of significant alcohol consumption [1]. In recent years, there has been increasing evidence that NAFLD by itself has important metabolic implications. Some authors refer to NAFLD as “insulin resistance associated steatosis” since all components of the metabolic syndrome correlate with liver fat accumulation independently of obesity [2]. NAFLD is becoming a risk factor for diabetes and cardiovascular diseases (CVDs) independently of insulin resistance, metabolic syndrome, plasma lipid levels, and other usual risk factors [3, 4]. NAFLD has also been shown to predict both type 2 diabetes and CVD independent of obesity. In addition, hepatic steatosis by itself is associated with a proatherogenic lipid profile and increased production of proinflammatory markers [5, 6].

The general association between NAFLD and CVD was established by the fact that the liver is involved in regulating/secreting numerous CVD risk factors, notably the cytokine tumor necrosis factor-alpha (TNF-α), an acute-phase protein CRP, glucose, lipoproteins, coagulation factors (plasminogen activator inhibitor-1), and a substance which increases blood pressure (angiotensin II) [7]. These authors claim that “hepatocytes are the last cells to be involved in the progressive chain of fat accumulation and probably the first cells to tell us that something is wrong”.

The exact pathogenesis of hepatic lipid accumulation seems to be very complex and only partially understood. As a whole, the general mechanism of liver fat accumulation involves an imbalance between lipid availability (from circulating lipid uptake or de novo lipogenesis) and lipid disposal (through fat oxidation or triglyceride-rich lipoprotein secretion) [8, 9]. Excessive fat accumulation in liver can occur as a result of increased fat delivery into the liver (dietary fatty acids and plasma nonesterified fatty acids derived from adipose tissue), increased fat synthesis in liver, reduced fat oxidation, and reduced fat exportation in the form of VLDL. Considering the complexity and heterogeneity of the mechanisms involved, it is quite difficult to imagine that it would be possible to identify a single gene variation as the single cause of the disease [10]. Body fat, insulin resistance, oxidative stress, mitochondrial dysfunction, cytokine/adipokine interplay, and apoptosis are potential risk factors of NAFLD [10].
One segment of the population particularly inclined to increased hepatic fat accumulation is postmenopausal women. Two thirds of postmenopausal women are considered overweight or obese and 43% present the metabolic syndrome [11]. Recent evidence indicates that menopause is indeed associated with the development of a state of hepatic steatosis [12]. Population-based studies indicate that nonalcoholic hepatic steatosis is more common in men than in women. However, following menopause there is a reversal in gender distribution so that NAFLD is more common in women. However, following menopause there is a reversal in gender distribution so that NAFLD is more common in women than in men [13]. In fact, it has been reported that nonalcoholic liver steatosis is twice as common in postmenopausal compared to premenopausal women, and that hormonal replacement therapy decreases the risk of steatosis [14]. Basic and clinical studies do support the hypothesis that estrogens protect from the development of NAFLD [15, 16]. In addition, alterations in body composition, fat distribution, and/or hormonal or metabolic changes that occur following menopause may influence the development and progression of NAFLD [17].

It seems that hormone replacement therapy decreases the risk of steatosis [14] as the prevalence of NAFLD is lower in postmenopausal women taking hormone replacement therapy than in women not taking it [18]. Nevertheless, although hormone replacement therapy appears safe in NAFLD, it is not recommended for liver protection because of the increased risk of cardiovascular events [19, 20]. In a recent review on NAFLD in older women, it was concluded that at present, there are no specific or effective pharmacological treatments available, and lifestyle modifications with weight loss and exercise are regarded as first line treatments [21].

The purpose of the present review is to present information relative to the development of a state of hepatic steatosis with estrogens withdrawal and the role of physical exercise to circumvent this phenomenon, emphasizing the contribution of the animal model on these issues.

2. Estrogens Withdrawal in Animals: Central and Peripheral Effects

2.1. Central (Extra-Hepatic) Effects of Estrogens Withdrawal. Ovariectomy (Ovx) in animals leads to increased food intake and body weight along with increased adipose tissue and liver fat accretion [22–24]. Data from observational and clinical trials support the fact that estrogens possess favourable metabolic effects as estrogens treatment has been shown to decrease body weight gain and fat accumulation in both animals and humans [25, 26]. In addition to increased food intake there is some evidence that energy expenditure is decreased with estrogens deficiency. A 40% reduction in ambulatory activity levels has been reported after ovariectomy in mice [27]. There are, therefore, central effects of estrogens withdrawal that are responsible for increased food intake and decreased energy expenditure resulting in adipocyte fat gain preferably in intra-abdominal region.

Since hyperphagia is a well-known response to Ovx and is prevented by estradiol replacement, many of the effects attributed to estradiol may be explained primarily by changes in food intake [28]. In fact, one view of Ovx-induced obesity in rat is that estrogens removal leads to a marked increase in body energy stores via increased energy intake and food efficiency along with decreased energy expenditure, which leads to increased energetic efficiency [24, 29]. This contributes to weight gain, especially as visceral or intra-abdominal fat, as observed in Ovx animals [30] and in women during and after menopause [31, 32]. Consequently, determinants of lipid metabolism such as liver triacylglycerol level and adipose tissue lipoprotein lipase (LPL) activity are altered in correspondence with an increased energy flux [29].

In other words, Ovx-induced increased energy efficiency is accompanied by concomitant adaptations of peripheral lipid metabolism that include the induction of pathways implicated in fat accumulation [23]. Therefore, the central effects of estrogens withdrawal on food intake and changes in insulin levels and its efficiency of action may indirectly affect liver fat accumulation in Ovx animals [24]. Central effects of estrogens supplementation in Ovx rats have been shown to lower food intake [33, 34], decrease adipose tissue LPL activity [35], and increase adipose tissue lipolysis [36], spontaneous physical activity [37], and energy expenditure [34, 38]. With regard to the central effects of estrogens, Picard et al. [24] postulated that Ovx induces obesity by removing the catabolic actions of estrogens, which act upon, yet poorly defined, central neuropeptidergic pathways that regulate energy balance. On the whole, there is little doubt that estrogens exert central effects that regulate feeding and energy expenditure through direct actions on the hypothalamus and/or through indirect actions by regulating adipose hormones such as leptin, adiponectin, and resistin [39].

2.2. Peripheral (Intrahepatic Effects) of Estrogens Withdrawal: Molecular Implications. In addition to the central effects, it is now well recognized that almost all tissues are under estrogenic influence in both men and women [40, 41]. Epidemiological and clinical evidence strongly suggest that estrogens, in particular 17β-estradiol, the most potent and dominant estrogens in mammals, play an important regulatory role in the metabolism and regional distribution of adipose tissue [42–44]. Estrogens promote subcutaneous fat depot after sexual maturation [43], while estrogens deficiency leads to increased fat, predominantly in visceral tissue [45]. It seems that estrogens control fat distribution by changing the lipolytic response distinctly into the two fat deposits, thus favouring fat accumulation in peripheral depots at the expense of the visceral depot [45]. There is also evidence that estrogens regulate LPL activity. It has been shown in several studies that ovariectomy in female rats results in increased adipose tissue LPL, while estrogens replacement decreased LPL activity [44].

In recent years, it has become evident that estrogens’ role in adipose tissue biology and lipid metabolism may be broader and more complex than initially appreciated. It seems that active metabolic tissues, such as the liver, are particularly sensible to estrogens effects in terms of different functions including lipid metabolism. The molecular and biological mechanisms underlying the metabolic actions...
of estrogens in liver are weakly understood. Estrogens are a steroid hormone mainly produced by ovaries whose actions are predominantly mediated by genomic mechanisms through its nuclear receptors (ER) α or β [46]. Outstanding advancements in recent years indicate that estrogens action in vivo is complex and often involves activation of cytoplasmic signalling cascades in addition to genomic actions mediated directly through estrogens receptors α and β. Estrogens may simultaneously activate distinct signalling cascades that function as networks to coordinate tissue responses [47]. These orchestrating distinct signalling pathways which involve specific complexes of cytoplasmic proteins might supplement or augment genomic effects of estrogens that are attributable to transcriptional activation by liganded receptors [48]. Therefore, it is not surprising that estrogens have been shown to exert rapid non-genomic biological actions through membrane bound subpopulations of ERs [49–51]. Interestingly, D’Eon et al. [52] reported novel genomic and non-genomic actions of estrogens that promote leanness in Ovx animals independently of reduced energy intake. In a recent review on estrogens regulation of adipose tissue functions, it was reported that estrogens reduce adiposity by promoting the use of lipid as fuel which is recognized by the activation of pathways that promote fat oxidation in muscle, by inhibition of lipogenesis in adipose tissue, liver, and muscle and by improved rates of adipocyte lipolysis [45]. The precise mechanism by which estrogens affect these functions is still unknown.

Estrogens-deficient state in ovariectomized animals has been repeatedly shown to result in a rapid liver fat accumulation [30, 53, 54]. Hepatic steatosis also developed in aromatase-deficient mice (ArKO; lacking the intrinsic ability to produce estrogens) and is diminished after treatment with estradiol [55]. Visceral obesity, metabolic syndrome with insulin resistance, as well as hepatic steatosis are the main features of the ArKO’s mouse phenotype [56]. Although many of the effects attributed to estrogens in the pathogenesis of Ovx-induced fat gain may be explained to a certain extent by the central effects of estrogens mostly via changes in food intake, D’Eon et al. demonstrated that estrogens reduced adiposity in Ovx rodents without confounding differences in food intake [52]. Their data are consistent with the phenotypes of both estrogens receptors-α (ERKO) knock-out and ArKO mice, both of which exhibit increased adiposity with no reported differences in food intake [38, 57–59]. Moreover, the results of Beckett et al. [60] suggest that estradiol regulates substrate metabolism in ectopic tissues such as skeletal muscles independently of changes in food intake. On the whole, it becomes evident that the ovarian hormonal status has important ectopic effects at the molecular level in peripheral tissues such as the liver rather than only central effects on food intake and energy expenditure. Accordingly, Fisher et al. [61] reported that despite a similar food intake, Ovx-pair fed animals gained markedly more weight than did Sham animals and nearly as much as Ovx-ad libitum animals. Likewise, data collected from our lab indicate that pair-feeding in Ovx rats does not completely prevent liver fat accretion in rats (Figure 1). Therefore, there must be factors other than food intake in the pathogenesis of liver fat accumulation in estrogen-deficient state.

Some intrahepatic pathways leading to lipid infiltration in estrogens deprived states have been investigated. Increased lipid uptake by liver as a result of increased fatty acid flow from circulation coming from intra-abdominal fat deposition attributed to the increased food intake after estrogens withdrawal can primarily and partially explain hepatic fat accumulation. The portal/fatty acid flux theory suggests that visceral fat, via its unique location and enhanced lipolytic activity, releases toxic-free fatty acids, which are delivered in high concentrations directly to the liver [62]. However, the portal/fatty acid flux theory has been questioned with the observation that the bulk of portal vein free fatty acids originate from subcutaneous adipose tissue in overnight-fasted obese individuals [63]. Nevertheless, there is little doubt that an increased arrival of lipids in situations of increased food intake and/or increased lipolysis contributes to liver lipid infiltration.

Disturbed regulatory mechanisms of lipids in the liver resulting from estrogens deficiency have been reported to play a role in liver fat accumulation. As estrogens levels decline, there may be increased lipogenesis and reduced fatty acid oxidation within the liver [17]. Liver de novo fatty acid synthesis that may result in hepatic steatosis is mostly regulated by three known transcription factors: SREBP-1c,
ChREBP, and PPAR-γ [64–66]. SREBP1-c activates fatty acid synthase (FAS) and stearoyl-CoA desaturase-1 (SCD-1) genes that are responsible for lipogenesis in liver [64]. D’Eon et al. [52] investigated the expression of several genes involved in the regulation of lipogenesis in the liver of Ovx and Ovx with estrogens replacement mice. Similar to their observations in adipose tissue, estrogens supplementation in Ovx rats decreased hepatic expression of the lipogenic gene SREBP-1c and its downstream targets ACC-1 and FAS compared to Ovx control rats. Similarly, increased lipogenesis in liver of Ovx rats was supported by changes in the expression and protein content of the lipogenic markers SREBP-1c and SCD-1 [67]. Furthermore, Na et al. [68] reported that estrogens deficiency in high-fat fed rats increased liver mRNA expressions of FAS and PPAR-γ while decreasing mRNA levels of the oxidative marker PPAR-α. In line with these reports, Ovx mice displayed visible steatosis even in a state of pair-feeding that was coincident with a remarkable elevation in hepatic PPAR-γ gene expression and downstream target genes FAS and ACC [68]. To confirm the role of estrogens in regulation of hepatic lipid metabolism, it has been shown that 17-beta-estradiol replacement in an animal model completely prevented the accumulation of lipids in the liver of Ovx rats and normalized the disturbed lipogenesis and lipid oxidation in liver [67, 69]. In addition to increased lipogenesis, a decrease in hepatic gene expressions involved in lipid oxidation, such as PPAR-α, was also reported in Ovx rats. PPAR-α is a receptor for peroxisome proliferators that functions as a lipid sensor that, when ineffective, can lead to reduced energy burning resulting in hepatic steatosis [70]. A decrease in PPAR-α in liver of Ovx rats has been reported [67]. Similar findings have also been reported in liver of ARKO mice [71]. Finally, Paquette et al. [72], using a physiological approach, reported a decrease of 34% in the rate of fatty acid oxidation in isolated hepatocytes of Ovx rats.

Besides lipogenesis and lipid oxidation, VLDL-TG production and secretion under low estrogenic condition might also be affected. In a study by Lemieux et al. [73] conducted on female Sprague-Dawley mice treated with the estrogens antagonist acolbifene, it was found that VLDL-TG secretion rate and microsomal transfer protein (MTP) mRNA levels were decreased by ~25–29%. Very recent data from our laboratory also revealed a decline in VLDL-TG production and MTP mRNA and protein content in Ovx rats [53]. Taken together, it is clear that estrogens withdrawal can have direct effects on hepatocytes and cellular constituents of liver tissue (intrahepatic effects) as well as central effects on food consumption, energy expenditure, and adipose tissue fat deposition that jointly contribute to the overall effects of liver fat accretion (Table 1).

3. NAFLD in Polycystic Ovary Syndrome (PCOS) Women

As mentioned above, premenopausal women are protected from the occurrence of CVD and NAFLD [16]. However, following menopause there is a reversal in gender distribution so that NAFLD becomes more common in women than in men [13]. It seems that a normal balance between androgens/estrogens ratio is required to maintain a proper distribution of body fat and normal metabolism in men and women [74]. For instance, hypoestrogenism in male rats and men is associated with fatty liver and features of the metabolic syndrome [16]. Similarly, hyperandrogenism in women is associated with increased central adiposity, insulin resistance, and increased risk of NAFLD [74]. Women with PCOS are at increased risk of metabolic syndrome and other complications such as type 2 diabetes and NAFLD [75, 76]. According to the European Society for Human Reproduction (ESHRE) and the American Society of Reproductive Medicine (ASRM), a diagnosis of PCOS requires two of the following three criteria: the presence of oligoovulation or anovulation, biochemical or clinical signs of hyperandrogenism, and the presence of polycystic ovaries [77]. Gambarin-Gelwan et al. [78] reported the presence of fatty liver in 55% of patients with PCOS, and nearly 40% of the patients diagnosed with NAFLD were lean. The beneficial effect of weight loss and exercise on liver fat accumulation of PCOS patients has been observed in a case report [79].

4. Exercise/Diet Interventions in Postmenopausal Women

More than 60% of American postmenopausal women are overweight or obese [80] and as mentioned earlier, it is well established that menopause is associated with weight gain, unfavourable alterations in body composition (elevated visceral fat deposition), and a state of hepatic steatosis [12, 81]. It seems that hormone replacement therapy (HRT) alleviates the metabolic consequences of menopause [82–84]. However, research on the safety of HRT is conflicting. The Women’s Health Initiative in the United States in 2002 and the Million Women Study in the UK in 2003 reported evidence of increased risk of heart disease, stroke, venous thromboembolism, and breast cancer with HRT in postmenopausal women [85, 86]. In general, although short-term use of HRT remains beneficial for severe menopausal symptoms, the uncertainty with the risks/benefits of HRT along with the well-publicized results of the above two large-scale HRT trials, has led to the conclusion that HRT will not protect future health in postmenopausal women [87]. Therefore, women continue to seek alternative options to improve their quality of life and reduce the risk of heart disease, osteoporosis, and breast cancer during postmenopause time [88].

Interestingly, the most research recommended cornerstone prevention/treatment for weight gain, elevated visceral fat deposition, and hepatic steatosis is weight loss through lifestyle interventions including exercise and/or diet. In a recent review, Zanesco and Zaros [89] reported that in an attempt to reduce the incidence of CVD in postmenopausal women, a variety of approaches has been used with conflicting results. Nevertheless, the change in lifestyle has been proposed as the most effective preventive action. This conclusion confirms the important role played by exercise and nutrition in the prevention and treatment of obesity, diabetes, and
CVD in postmenopausal women [90, 91]. Data from a 5-year randomized clinical trial known as the Women's Healthy Lifestyle Project had previously demonstrated that weight gain and increased waist circumference during the peri- to postmenopausal period can be prevented by a long-term lifestyle dietary and physical activity intervention [92].

One of the most important components of lifestyle is physical activity which has been known for a long time to be a powerful low-risk mean for the promotion of all aspects of human health including menopause [93]. Postmenopausal women might demonstrate a greater response to exercise since it was shown that even small increases in physical activity and exercise at the time of menopause can help prevent the atherogenic changes in lipid profiles and the weight gain experienced by these women [91]. Longitudinal and cross-sectional studies have shown that physical activities, such as moderate-intensity sports/recreational activity or biking and walking for transportation are associated with lower body fat and less central adiposity in postmenopausal women [81, 94, 95]. Moderate-intensity exercise (walking or 45 min moderate-intensity aerobic activity 5 d/wk) can also result in improvements in coronary/metabolic risk factors such as insulin resistance in postmenopausal women [96–98]. The results of a study by Hagberg et al. [99] even indicated that numerous years of high-intensity endurance training had a greater effect on total and regional body fat values than HRT in postmenopausal women. Given that obesity is extremely prevalent and difficult to treat, prevention of weight gain after menopause is an important health target. A successful model of weight gain prevention has yet to be established [100]. In a longitudinal study, Hagmar et al. [101] reported that former elite but still active endurance female athletes had higher flow-mediated vasodilatation, used as an indicator of endothelial function, than control subjects. This latter study does not, however, discriminate the previous exercise training conducted during the reproductive period from the training conducted during menopause. A response to this question may be tentatively obtained from a recent study in Ovx animals in which it was found that to be effective in reducing adipoocytes and liver fat accumulation, exercise must be conducted concurrently with estrogens withdrawal [102]. On the whole, it seems that postmenopausal women with high levels of physical activity have lower body and abdominal fat and are less likely to gain fat (total and abdominal) during menopause than those with lower levels of physical activity [81]. Endurance training has been reported to be very effective in reducing intrahepatic triglycerides content in human (for a recent review see [103]). More recently, a 12-month intensive lifestyle intervention in patients with type 2 diabetes has been reported to reduce hepatic steatosis by as much as 25% [104]. However, information is lacking on the role of exercise training specifically on prevention and/or reversal of hepatic steatosis in postmenopausal women.

5. Exercise in the Animal Model of Menopause

Ovx animals can benefit from an exercise training program with a reduction in fat gain [105]. In 2002, Shinoda et al. [106] showed that exercise training exerts a strong action upon reduction in body fat accumulation following a decrease in estrogens levels. In spite of the reduction in body fat, 8 wk of endurance exercise training in this study did not reduce overall weight gain suggesting a compensatory increase in muscle weight by training. This is an interesting asset of exercise since food restriction protocols in Ovx

| Table 1: Summary of the central and intrahepatic effects resulting in liver fat accumulation with estrogens withdrawal. |
|---|
| Central effects | Intra-hepatic effects |
| CNS/hypothalamic effects | Lipid uptake |
| (i) Food consumption | (i) Unknown (possible mechanism of upregulation of fatty acid uptake via estrogens-dependent pathways, yet to be explored) |
| (ii) Leptin secretion | Lipogenesis |
| (iii) Activity and energy expenditure | (ii) SREBP-1c and PGC1α |
| Lipid profile and adipose tissue effects | (iii) SCD-1 |
| (i) Absence of estrogens causes fat redistribution/gain particularly increased intra-abdominal fat and altered lipid homeostasis (portal/fatty acid flux theory) | (ii) FAS |
| | (iv) ACC |
| | (v) PPAR-γ |
| | Lipid oxidation |
| | (i) PPAR-α |
| | (ii) HSL |
| | (iii) Fatty acid β-oxidation |
| | VLDL-TG production and secretion system |
| | (i) VLDL-TG production in Ovx rats |
| | (ii) MTP and DGAT2 |

CNS: central nervous system; SREBP-1c: sterol-regulatory-element-binding-protein 1c; PGC1α: peroxisome proliferator-activated receptor gamma coactivator-1 alpha; SCD-1: stearoyl-CoA desaturase-1; FAS: fatty acid synthase; ACC: acetyl-CoA carboxylase; PPAR-α, -γ: peroxysome proliferator-activated receptor-alpha, -gamma; HSL: hormone-sensitive lipase; VLDL-TG: very low density lipoprotein-triglyceride; MTP: microsomal triglyceride transfer protein; DGAT2: diacyl-glycerol acyltransferase-2.
rats have been known to be associated with a decrease in muscle mass [107]. In this regard, it was shown that muscle tissue hypertrophy induced by a progressive loading exercise program has a stimulatory effect on bone mass in Ovx rats [108].

A major concern of a reduction in estrogenic status is insulin resistance. It is well known that environmental factors such as aging, obesity, and physical inactivity are linked to the development of a state of insulin resistance and type 2 diabetes mellitus. Rationally, the prevalence and progression of type 2 diabetes are likely to increase in postmenopausal women. Several studies reported insulin resistance in experimental animals after Ovx, which can be reversed by HRT and exercise training although the results have been somewhat conflicting [22, 109, 110]. One of the best evidence of the effects of exercise training on estrogens withdrawal-induced insulin resistance comes from the study of Saengsirisuwan et al. [111]. These authors showed that ovariectomy in female Sprague-Dawley rats resulted in the development of a systemic metabolic condition presenting the characteristics of the metabolic syndrome including increased visceral fat content, abnormal serum lipid profile, impaired glucose tolerance, and defective insulin-mediated skeletal muscle glucose transport. Saengsirisuwan et al. [111] also provided evidence that whole-body and skeletal muscle insulin resistance is effectively corrected by endurance exercise training alone and estrogens replacement alone. Despite this and similarly to Choi et al. [112], they could not find evidence that exercise training additively modulates insulin action in Ovx animals that also received estrogens replacement, suggesting that endurance exercise training and estrogens may share common mechanisms to correct defects in ectopic tissues caused by estrogens deficiency. This concept is supported by the observations that transcripts encoding estrogens signalling in skeletal muscle, cardiac muscle, and liver are enhanced by regular exercise [113–115].

As previously mentioned, menopause is associated with the development of a state of hepatic steatosis [12, 116], which plays an important role in the development of insulin resistance [117]. Ectopic fat in liver may be even more important than visceral fat in the characterization of metabolic obesity in humans [118, 119]. An alternative to counteract liver fat accumulation with estrogens withdrawal may be exercise training. It has been reported that exercise training prevents fat accumulation in livers of high-fat fed rats [120, 121]. Recently, we reported evidence that endurance exercise training conducted concurrently with estrogens withdrawal did prevent liver fat accumulation in rats [102]. This latter study is particularly interesting since it also showed that if exercise is conducted only before the ovariectomy, there was no protective effect of exercise on subsequent Ovx-induced liver fat accumulation. On the other hand, if exercise was started at the same time as Ovx was performed, liver fat accumulation was prevented emphasizing the finding that exercise must be conducted concurrently as estrogens withdrawal to be effective. To explore mechanisms by which exercise prevents liver fat accumulation in Ovx rats, Pignon et al. [122] conducted a subsequent study in which they measured the expression of several genes in liver. They found that exercise training acts as estrogen supplementation in properly decreasing several genes of lipogenesis (SREBP-1c, ChREBP, SCD-1, ACC) as well as decreasing several biomarkers of subclinical inflammation (IL-6, NFκB, TNFα) in Ovx rats.

6. Resistance Training (RT) in Postmenopausal Women

Weight loss achieved through restrictive diets often results in negative effects on muscle mass [123]. In this regard, resistance training seems to be a logical choice considering its beneficial effects on muscular strength in postmenopausal women [124]. It has been demonstrated that RT exercise can be an effective substitute for hormone replacement therapy in preventing menopausal-related osteoporosis and sarcopenia [125]. In addition to increasing muscle mass and improving muscle function, RT has been reported to induce decreases in total and abdominal fat [126, 127]. On the other hand, there are studies that showed no reduction in fat tissue with RT exercise [128]. Eight weeks of low intensity, short duration RT program was not sufficient to produce significant modifications in body composition and blood lipid concentrations in postmenopausal women, although it produced substantial improvements in muscle strength [129]. In obese sedentary postmenopausal women, it has been suggested that RT has the potential to ameliorate/prevent the development of insulin resistance and may reduce the risk of glucose intolerance and non-insulin-dependent diabetes mellitus [130]. In these subjects, RT alone or in combination with a weight loss program (diet) (RT+WL) improved muscular strength and insulin action and glucose homeostasis. However, the same authors in a subsequent study showed that body weight and fat mass did not change with RT alone, but decreased with RT+WL [131]. Nevertheless, RT and RT+WL both increased fat-free mass and resting metabolic rate in postmenopausal women [132]. Considering the fact that subjects in RT group were nonobese and subjects in RT+WL group were obese postmenopausal women, the authors suggested that RT may be a valuable component of an integrated weight management program in postmenopausal women. In a recent study conducted in overweight and obese postmenopausal women, it was reported that RT combined to caloric restriction was more effective that caloric restriction alone in reducing fat mass (%) and trunk fat mass [133]. Although, as for endurance training, there is a paucity of information on the impact of RT on NAFLD in postmenopausal, it seems that RT constitutes an asset to overcome several of the deleterious metabolic effects associated with menopause.

7. Resistance Training (RT) in Ovx Animals

In Ovx rats, Corriveau et al. found that an 8 wk program of resistance training in conjunction with restrictive diet reduced intra-abdominal fat depot and plasma-free fatty acid levels and prevented liver fat accumulation [54]. It was concluded that RT is an asset to minimize the deleterious
effects of ovarian hormone withdrawal on abdominal fat and liver lipid accumulation in Ovx rats. Leite et al. [134] also indicated the potential benefits of resistance training as an alternative strategy to control the negative effects of ovariectomy. Twelve weeks of strength training in Ovx rats decreased fat content in liver, skeletal muscle, and intra-abdominal adipose tissue and positively changed lipid profile such as increasing HDL levels while decreasing total cholesterol and LDL levels. In both of these studies, the RT program consisted of climbing a vertical grill with weights attached to the tail of rat.

Using the same design as Corriveau et al. [54], Pighon et al. conducted two studies on liver and body fat regain in Ovx rats using resistance training. In a first study [135], they tested the hypothesis that substituting food restriction by resistance training after a period of weight loss would maintain the decrease in fat accumulation in liver and adipose tissue that occurs with weight loss. We found that cessation of an 8 wk food restriction regimen aimed at lower body weight and fat accumulation in Ovx rats may be substituted by a resistance training program (over 5 more weeks), without causing any appreciable regain of fat in liver and adipose tissue. Our group suggested that changing from a food restriction regimen to a resistance training program may be an interesting strategy to promote successful long-term weight reduction in postmenopausal women. In a second study, Pighon et al. [136] investigated the effects of maintaining RT or food restriction on body weight regain, fat mass, and liver lipid infiltration in Ovx animals previously submitted to a food restriction + RT weight loss program. We observed that maintaining only food restriction was the most effective but that maintaining RT alone was an asset to attenuate intra-abdominal and liver fat reincrease. Again we suggested that the maintenance of only one component of an RT+ food restriction weight loss program constitutes a positive strategy to reduce body weight and fat mass relapse in postmenopausal women.

8. Exercise and Weight Regain

It seems that maintenance of weight loss is a core problem in the treatment of obesity, and long-term maintenance of weight loss remains a challenge. A common treatment for weight loss is food restriction or hypocaloric diet therapy. Although interventions aimed at weight loss are well supported [137], reductions in weight by dietary restriction are typically modest and are increasingly viewed as an unsustainable outcome of lifestyle modification [138, 139]. It thus seems that there is a high rate of recidivism after diet-induced weight loss. One of the main underlying problems in this matter appears to be the compensatory metabolic responses to weight reduction which results in a strong drive to regain lost weight [140, 141]. Such responses include enhanced metabolic efficiency with a progressively increasing appetite along with interrelated alterations like improved insulin sensitivity and energetically favourable shift in fuel utilization characterized by an increased preference for carbohydrate oxidation at the expense of lipid oxidation which may explain why successful weight reduction is so hard to achieve [140]. MacLean et al. [142] believe that these compensatory metabolic adjustments are part of an interrelated group of adaptations in the homeostatic feedback loop between the periphery and the central nervous system that controls body weight. It seems that the homeostatic feedback system defending body weight and adiposity is fundamental to the metabolic drive to regain lost weight [142, 143]. The positive aspect is that modification of this biological predisposition is possible. Interestingly, exercise training seems to positively alter this propensity and has been shown to be important to successful weight maintenance after weight loss programs [144, 145]. Levin et al. [146, 147] reported that regular physical activity lowers the defended level of weight gain and adiposity without compensatory increase in intake and with a favourable alteration in the development of the hypothalamic pathways controlling energy homeostasis as compared to calorically restricted rats. These authors suggested that exercise produces a different set of regulatory signals from caloric restriction that resets the homeostatic balance between energy intake and expenditure toward defence of a lower level of weight gain and adiposity.

Body weight and fat mass gain and regain following weight loss may be even more critical after menopause since physiological withdrawal of ovarian hormones, by itself, negatively affects the energy balance. Similarly to the above discussion on weight regain, Nicklas et al. [148] suggested that the poor success rate of food restriction treatment in postmenopausal women may be due in part to metabolic adaptations that occur in response to a long period of negative energy balance such as declined fat oxidation, resting metabolic rate, and adipocyte lipolytic responsiveness which predispose the regain of body weight. These authors showed that the addition of endurance exercise to diet-induced weight loss program minimizes these negative metabolic adaptations in postmenopausal women. Similarly, substituting a walking training to a very-low-energy diet in premenopausal obese women improved maintenance of losses in weight and waist circumference and prevented further clustering of metabolic risk factors [149]. As mentioned above, data on Ovx rats suggest that changing from a food restriction regimen to an exercise training program can be an interesting strategy to promote long-term weight reduction in postmenopausal women [135].

9. Conclusion

It is becoming increasingly clear that once women reach menopause in their life they are exposed to increasing risks of developing complications due to a decrease in estrogens-related protective effects. Among the protective effects of estrogens, liver fat accumulation seems to be of primary importance due to its important role in the development of insulin resistance, atherosclerosis, and cardiovascular diseases. Information relative to cellular and molecular mechanisms using the animal model indicates that gene expressions of molecules such as SREBP-1c, ChREBP, and SCID-1 involved in the lipogenesis pathway are upgraded with estrogens withdrawal, while molecular markers of the oxidative pathway including CPT-1 and PGC-1 and
molecular markers of VLDL production such as MTP and DGAT-2 are reduced with estrogens loss [33, 122]. These observations point to the direction that a reduction in estrogens production results in central effects such as an increase in food intake and a reduction in energy expenditure, but may also metabolically affect tissues such as the liver thus resulting in ectopic fat accumulation. In a recent review paper on NAFLD in older women [21], it was concluded that there is no effective pharmacological treatment available and that lifestyle modifications with weight loss and exercise are regarded as first line treatment. Although there is a lack of information on the role of exercise on liver lipid infiltration during the premenopausal to postmenopausal transition, data on the animal model clearly indicate that exercise training exerts a powerful action in reducing liver fat accumulation especially if exercise training is conducted at the same time as estrogens withdrawals [102, 120]. Exercise training seems to exert an estrogenic-like effect not only on expression of genes involved in lipid accumulation but also on expression of genes of subclinical inflammation in liver [122]. Taken together, it seems that if there is a time in women’s life where physical exercise is important, it is with menopause.

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Review Article

Dietary Conjugated Linoleic Acid and Hepatic Steatosis: Species-Specific Effects on Liver and Adipose Lipid Metabolism and Gene Expression

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Objective. To summarize the recent studies on effect of conjugated linoleic acid (CLA) on hepatic steatosis and hepatic and adipose lipid metabolism highlighting the potential regulatory mechanisms.

Methods. Sixty-four published experiments were summarized in which trans-10, cis-12 CLA was fed either alone or in combination with other CLA isomers to mice, rats, hamsters, and humans were compared.

Summary and Conclusions. Dietary trans-10, cis-12 CLA induces a severe hepatic steatosis in mice with a more muted response in other species. Regardless of species, when hepatic steatosis was present, a concurrent decrease in body adiposity was observed, suggesting that hepatic lipid accumulation is a result of uptake of mobilized fatty acids (FA) from adipose tissue and the liver’s inability to sufficiently increase FA oxidation and export of synthesized triglycerides. The potential role of liver FA composition, insulin secretion and sensitivity, adipokine, and inflammatory responses are discussed as potential mechanisms behind CLA-induced hepatic steatosis.

1. Introduction

Obesity is a chronic metabolic nutritional disorder that has increased at an alarming rate in the last 20 years [1]. In the US, 68% of the adults (age ≥ 20 years) and 18% of children (2–19 years) are either obese or overweight as per the National Health and Nutrition Examination Survey [2, 3]. Incidence of obesity is associated with many health complications such as hypertension, hyperlipidemia, cardiovascular disease, type 2 diabetes [4], and a range of lipid abnormalities, the most common being nonalcoholic fatty liver disease (NAFLD) [5].

NAFLD is an important health concern due to its high prevalence (~20% of adult population) and its association with insulin resistance and metabolic syndrome [5]. It is characterized by hepatic lipid accumulation primarily in the form of triglycerides (TG) [6]. Some of the potential steps involved in the progression of NAFLD may involve increased uptake of circulating fatty acids (FA) [7], increased hepatic denovo lipogenesis (DNL) [8], reduced rate of FA oxidation [9], or reduced FA secretion [10, 11]. When NAFLD is associated with inflammation and fibrosis, it is termed as nonalcoholic steatohepatitis (NASH), a serious condition that could lead to liver cirrhosis, hepatic carcinoma, and liver failure [12]. The pathogenesis of NAFLD can be explained by “two hit” hypothesis suggesting steatosis as the “first hit” which increases the vulnerability of liver to various second hits like oxidative stress and inflammation leading to NASH [13].

Although no specific guidelines exist for treatment of NAFLD, recommendations are aimed at reducing body weight due to its strong association with obesity and metabolic syndrome [4]. In this regard, bioactive lipids/FA as functional food may be important in modulating metabolism and body weight. A specific group of polyunsaturated FA collectively known as conjugated linoleic acid (CLA) have been suggested to have an effect on regulating energy metabolism [14] and is being used commercially as a weight-loss supplement. CLA were recently granted “Generally Recognized As Safe”, status in the United States (GRN no. 232; http://www.cfsan.fda.gov/) for use as a dietary
supplement. However, CLA effects are varied depending on the type of CLA isomer, the animal’s physiological condition, and the tissue type examined. In this paper, we summarize the recent studies on effect of CLA on hepatic lipid metabolism highlighting the potential regulatory mechanisms.

2. Conjugated Linoleic Acid

Conjugated linoleic acid refers to a group of dienoic derivatives of linoleic acid with conjugated double bonds arranged in different combinations of cis and trans configuration [15]. Currently, 16 naturally occurring CLA isomers have been identified with different positional (7/9, 8/10, 9/11, 10/12, and 11/13) and geometric (cis/cis, trans/trans, cis/trans, and trans/cis) combinations [16, 17].

Sources of CLA include those naturally present in dairy products and meat from ruminant animals or those contained in industrially hydrogenated vegetable oils and other synthetic products [14]. The CLA originating from the ruminant products predominantly consist of cis-9, trans-11 CLA (>80%), with a small amounts of trans-10, cis-12 CLA and other isomers [18]. The industrially synthesized CLA and other commercial products intended for human consumption typically consists of equal amounts of cis-9, trans-11 CLA and trans-10, cis-12 CLA and other isomers [19]. Of all the CLA isomers, cis-9, trans-11 CLA and trans-10, cis-12 CLA have been the most widely studied due to their biologically active properties [15].

3. Physiological Effects of CLA

A great deal of current interest in CLA is due to their bioactive properties including anticarcinogenic [19], antiatherogenic [20], immunity enhancing [21], and effects on body composition [22]. Each CLA isomer has unique bioactive properties, and hence, the biological effect from a mixture of dietary CLA isomers, as is the case in most of the studies, would be the combined effect of their distinct isomers [15]. For example, cis-9, trans-11 CLA and trans-10, cis-12 CLA have additive effects on cancer [23] and immune cell functions [24] but are antagonistic on insulin sensitivity. While cis-9, trans-11 CLA improves insulin sensitivity, trans-10, cis-12 CLA causes insulin resistance. Also, trans-10, cis-12 CLA is solely responsible for changes in body composition and reducing adipose mass [25].

3.1. Body Weight and Lean Mass. CLA reduces body weight and body fat mass and increases lean mass in different species [22]. However, the response appears to vary depending on species, physiological stage, and fat depot [22, 26]. Table 1 provides a summary of studies reviewed across species with respect to body weight and adiposity, where the number of experiments showing significant (P > 0.05) increases, decreases, or no change and the mean response to dietary trans-10, cis-12 CLA within those categories are reported. The range of trans-10, cis-12 CLA addition in these studies varied between 0.1 and 1 percent of the diet.

Trans-10, cis-12 CLA reduces body fat to a maximum extent in mice (60% to 80%) [27, 28]. However, modest and inconsistent effects are seen in rats [49, 82], hamsters (9% to 58%) [54, 55], and pigs (6% to 25%) [83]. Similarly, variable responsiveness to CLA was observed for epididymal, perirenal, and subcutaneous body fat depots [55]. Inconsistent responses to trans-10, cis-12 CLA have been reported in clinical trials with humans [84]. Some have shown significant effects on body composition [63, 85], while others have not [64, 65]. The differences in the responses are attributed to differences in the dose levels, age, and rate of adipose tissue TG turnover [14, 66, 84]. The response to CLA isomers also depends on the physiological state of the animal which is probably due to differences in the preferential uptake of CLA by different tissues. For example, trans-10, cis-12 CLA is preferentially taken up by the mammary tissue during lactation leading to substantial (~45%) decrease in milk lipid synthesis [29].

3.2. Effects of CLA on Hepatic Lipid Metabolism. Liver plays an important role in energy homeostasis, as it converts excessive dietary glucose into FA which is exported as TG. Liver is an important target for CLA effects irrespective of the physiological condition. Of the different CLA isomers, trans-10, cis-12 CLA causes increased lipid accumulation leading to hepatic steatosis [30–32, 86]. However, the intensity of lipid accumulation varies depending on the level of CLA in the diet, duration of feeding, physiological condition, and animal species (Table 1). The factors leading to hepatic lipid accumulation are multifactorial involving increased FA influx, increased FA synthesis, and altered FA oxidation and TG secretion insufficient to prevent lipid accumulation (Figure 1) [33]. These mechanisms are probably not mutually exclusive and could act in a coordinated manner to hasten the development and progression of fatty liver [87].

3.2.1. Hepatic FA Synthesis. Under normal conditions, de novo lipogenesis contributes minimally to the lipid pool in the liver [88]. However, the lipid synthesis increases to as much as 26% during steatotic conditions [89]. The increase in hepatic lipid content due to CLA, specifically trans-10, cis-12 CLA, is commonly associated with increased hepatic lipogenesis [30]. In mice, CLA has been repeatedly shown to increase the expression of sterol regulatory element-binding protein-1c (SREBP-1c), key transcriptional regulator in hepatic lipogenesis and its downstream genes acetyl CoA carboxylase (ACC), fatty acid synthase (FASN), and stearoyl CoA desaturase-1 (SCD1) [30, 34, 35] (Table 2). However, in rats and hamsters, the responses are equivocal. The increase in SREBP-1c expression in mice is attributed to hyperinsulinemia (Figure 1) [30]. The decreased expression of lipogenic (ACCl, ACC2, FASN, and SCD1) genes in the absence of insulin in mice fed trans-10, cis-12 CLA further supports this argument [33]. In addition to SREBP-1c, insulin induces the expression of peroxisome proliferator-activated receptor-y (PPAR-y) [90], which is in low abundance under normal conditions [91]. PPAR-y expression is increased in steatotic liver (Figure 1) [30, 92], while its
Table 1: Studies showing that trans-10, cis-12 CLA induced significant ($P < 0.05$) increases or decreases, or where there was no change ($P > 0.05$) in body, adipose, and liver weights and liver lipid concentration.

| Species | Change | Body weight | Adipose tissue | Liver weight | Liver lipids |
|---------|--------|-------------|----------------|--------------|-------------|
| Mice$^1$ | Increase | — | — | 24 (92) | 19 (515) |
| | Decrease | 21 (31)$^2$ | 29 (666) | — | — |
| | No change | 16 | — | 2 | 2 |
| | Increase | — | — | — | 1 (25) |
| Rats$^3$ | Decrease | — | 1 (23) | — | 4 (19) |
| | No change | 11 | 3 | 8 | 4 |
| | Increase | — | — | 8 (20) | — |
| Hamsters$^4$ | Decrease | 2 (14) | 11 (20) | — | 3 (37) |
| | No change | 11 | 2 | 2 | 5 |
| | Increase | — | — | — | — |
| Humans$^5$ | Decrease | 2 | 6 | — | — |
| | No change | 11 | 13 | — | — |

$^1$Studies used: [22, 25, 27–48].
$^2$Number of observations (mean percent change).
$^3$Studies used: [49–53].
$^4$Studies used: [54–62].
$^5$Studies used: [63–81].

Figure 1: Current concepts in the pathways of trans-10, cis-12 CLA-induced hepatic steatosis. (1) Adipose tissue lipodystrophy caused by increased proinflammatory cytokines and reduced adipokines leading to higher circulatory levels of free FA (FFA). (2) Hyperinsulinemia induced by systemic insulin resistance. (3) Alterations in hepatic lipid metabolism leading to hepatic steatosis. (4) Alterations in hepatic FA composition. SREBP-1c, Sterol regulatory element-binding protein-1c; PPAR-γ, peroxisome proliferator activated receptor-γ; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; IL-8, interleukin-8; PEPCK, phosphoenol pyruvate carboxykinase; G6P, glucose 6-phosphatase; ChREBP, carbohydrate response element-binding protein; PPAR-α, peroxisome proliferator-activated receptor-α; LC-PUFA, long chain polyunsaturated FA.
ablation ameliorates the condition in mice [93]. Insulin resistance in response to trans-10, cis-12 CLA could upregulate genes of glucogenic pathway (e.g., PEPCK, G6P) leading to hyperglycemia (Figure 1) [94]. In turn, elevated blood glucose concentrations could upregulate hepatic lipogenesis through carbohydrate response element binding protein (ChREBP), a transcriptional regulator modulated by glucose (Figure 1). The targeted deletion of ChREBP in the liver improves the steatotic conditions in ob/ob mice [94]. However, the role of ChREBP in CLA-induced hepatic steatosis is not known. Although hyperinsulinemia triggers the hepatic lipogenesis, CLA-induced hepatic steatosis in the absence of insulin suggests the involvement of other regulatory mechanisms affecting hepatic lipid accumulation [33].

3.2.2. Hepatic FA Uptake and TG Secretion. In mouse experiments, dietary trans-10, cis-12 CLA was associated with upregulation of genes associated with FA uptake and TG secretion (FAT/CD36; Table 2). During hepatic steatosis about 59% of hepatic TG is derived from free FA released from the adipose tissue and 15% is derived from dietary fat [89]. FA transporters, (FATP5, FAT/CD36, FABP-1, FABP-4, and FABP-5) regulate the FA uptake by hepatocytes. While the overexpression of these proteins promotes steatosis, functional deletion ameliorates the condition [98–100]. As CLA are natural ligands and activators of PPAR-γ [101] the upregulation of FAT/CD36 by trans-10, cis-12 CLA [32, 33, 102] could be through PPAR-γ leading to increased hepatic FA uptake. In addition to FAT/CD36, we have observed modest increases in the expression of FABP-1 (1.39 fold) and FABP-2 (1.7 fold) in liver of lactating mice fed trans-10, cis-12 CLA (Kadegowda, A. K. G., Erdman, R. A., and Loo, J. J., unpublished results).

Besides enhanced FA uptake and lipogenesis, alteration in very low-density lipoprotein (VLDL) secretion rates could also result in liver fat accumulation [103]. The VLDL production and secretion is increased in response to elevated lipid concentrations. However, impaired or insufficient fat export via VLDL predisposes animal to hepatic steatosis [10]. Trans-10, cis-12 CLA reduced TG secretion leading to higher lipid accumulation in HepG2 cells due to reduced apolipoprotein B synthesis [104]. Conversely, lipoprotein clearance was not affected in mice fed CLA [31, 102]. The TG export was increased with higher rate of VLDL secretion; however, it was insufficient to eliminate increased FA flux entering the liver leading to hepatic steatosis [31].

3.2.3. Hepatic FA Oxidation. Hepatic FA oxidation encompasses β-oxidation in mitochondria and peroxisomes and ω-oxidation in the microsomes [105]. The FA < C8 to C20 are catabolized through the mitochondrial β-oxidation pathway, while FA > C20 are initially catabolized in the peroxisomes to shorter FA which are then shuttled to mitochondria for further oxidation [32]. Previous studies have reported variable responses in hepatic FA oxidation with trans-10, cis-12 CLA. Most of the studies have shown increased FA oxidation [27, 34, 36, 56, 106], while some have reported reduced [32] or unaltered FA oxidation [22] with CLA.

### Carnitine palmitoyltransferase-1 (CPT1)

Carnitine palmitoyltransferase-1 (CPT1) is the rate limiting enzyme for mitochondrial β-oxidation pathway, as it regulates the transport of fatty acyl CoA into mitochondria. When measured in mice, CPT1 gene expression was consistently increased by CLA (Table 2) which might be mediated through transcriptional regulator PPAR-α as it regulates the key enzymes (e.g., CPT1, CPT2, and ACO) involved in hepatic FA oxidation [50].

Despite increased FA oxidation hepatic steatosis was consistently observed in mice (Tables 1 and 2). Since studies showing increased FA oxidation were also associated with increased hepatic lipogenesis, it is possible that that the rates of hepatic lipogenesis far exceed the rates of FA oxidation resulting in increased lipid accumulation. Along with increased lipogenesis the level of malonyl CoA, a product of ACC, was also increased that allosterically inhibits CPT1 enzyme activity [36]. Thus, despite higher expression of FA oxidation genes, it is possible that FA combustion might be depressed in vivo leading to steatosis.

Some studies have shown CLA induced downregulation of genes related to mitochondrial β-oxidation (CPT1), and ω-oxidation (cyc P450 and FMO3) [32]. We have also observed decreased expression of CPT1, ACOX1, and FMO3 without any changes in hepatic lipogenic genes of lactating mice fed trans-10, cis-12 CLA (Kadegowda, A. K. G., Erdman, R. A., and Loo, J. J., unpublished results). The variable responses among different studies can be attributed to the level and type of fat used in the experimental diet along with the physiological conditions of animal used in the experiment.

3.2.4. Effect of CLA on Hepatic FA Composition. Trans-10, cis-12 CLA-induced hepatic steatosis is characterized by changes in hepatic FA composition [29, 37, 107–111] similar to those induced during NAFLD [112]. The hepatic FA composition in steatotic liver determines the extent of susceptibility of liver injury [113]. The steatotic liver FA profile is characterized by substantial reductions in long chain polyunsaturated FA (LC-PUFA) concentrations; specifically that of arachidonic acid (C20:4n-6). While linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) are unaltered, the concentrations of eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) are decreased. The desaturation and elongation of linoleic and α-linolenic acid by desaturases (Δ^6-desaturase, Δ^5-desaturase) and elongases (ELOVL-2, ELOVL-3) are involved in synthesis of LC-PUFA. Trans-10, cis-12 CLA inhibits both Δ^5- and Δ^6-desaturase in HepG2 cells [114]. A recent tracer study with [U-13C]linoleic acid showed significant reduction in n-6 PUFA synthesis by inhibition of elongation and desaturation in the liver homogenates of neonatal pigs [115]. A decrease in arachidonic acid synthesis would alter eicosanoid metabolism and potentially reduce the synthesis of prostaglandin E2 (PGE2) [116] which is known to have protective effects on liver [117].

Typical NAFLD is also characterized by increased n-6 : n-3 LC-PUFA ratio which favors lipid synthesis over lipid oxidation and secretion leading to hepatic lipid accumulation [118]. Trans-10, cis-12 CLA reduces the n-3 PUFA in liver [38, 109] in addition to arachidonic acid. The
Table 2: Studies showing that trans-10, cis-12 CLA induced significant ($P < 0.05$) increases ($\uparrow$), decreases ($\downarrow$), or no change (↔) ($P > 0.05$) in hepatic gene expression and circulating levels of insulin, adipokines, and TNF-α. Genes are classified based on their ascribed function.

|                  | Mice1 |  |  | Rats2 |  |  | Hamsters3 |  |  |
|------------------|-------|---|---|-------|---|---|-----------|---|---|
| Lipogenesis      |       |   |   |       |   |   |           |   |   |
| ACC              | 5 (126)$^4$ |     | 1 |       |     | 1 (99) |       |     | 1 |
| FASN             | 7 (243) |     | 1 |       |     | 2 |       |     | 2 |
| SCD1             | 2 (150) |     | 3 |       |     | 4 |       |     | 3 |
| SREBP-1c         | 3 (53)  |     | 2 |       |     | 2 |       |     | 2 |
| PPAR-γ           | 2 (200) |     | 1 |       |     | 1 |       |     | 1 |
| ME               | 5 (205) |     | 1 |       |     | 1 |       |     | 1 |
| FA uptake, secretion, and oxidation |       |   |   |       |   |   |           |   |   |
| CPT1             | 4 (107) | 1 (59) | 1 |       |     | 4 |       |     | 2 |
| ACO              | 5 (117) |     | 1 | 2 (130) |     | 4 |       |     | 2 |
| PPAR-α           | 1 (53)  |     | 1 | 1 (125) |     | 4 |       |     | 3 |
| FAT/CD36         | 3 (533) |     | 1 |       |     | 2 |       |     | 2 |
| LPL              | —      | — | — |       |     | 1 |       |     | 1 |
| Insulin          | 12 (2492) | 1 (29) | 3 |       |     | 3 |       |     | 1 |
| Adiponectin      | —      | 6 (77) | 5 |       |     | 4 |       |     | 3 |
| Leptin           | —      | 10 (71) | 2 |       |     | 1 |       |     | 1 |
| TNF-α            | —      | 4 (32) | 1 | 1 (44) |     | 1 |       |     | 1 |

1Studies used: [27, 29–35, 45, 95, 96].
2Studies used: [49–52, 97].
3Studies used: [54, 56, 59, 61].
4Number of observations (mean percent change).

ACC: acetyl CoA carboxylase, FASN: fatty acid synthase, SCD1: stearoyl CoA desaturase-1, SREBP-1c: sterol regulatory element-binding protein-1c, PPAR-γ: peroxisome proliferator activated receptor-γ, ME: malic enzyme, CPT1: carnitine palmitoyl transferase 1, ACO: acyl-CoA oxidase, PPARα: peroxisomal proliferator activated receptor α; FAT/CD36: fatty acid translocase, LPL: lipoprotein lipase.

n-3 PUFA downregulate SREBP-1c and upregulate PPAR-α, which regulates lipid oxidation (CPT1, ACOX1) and secretion (ApoB100). A decrease in hepatic n-3 PUFA would not only reduce lipid oxidation but increase lipogenesis leading to hepatic steatosis [118]. Although the trans-10, cis-12 CLA-induced responses in FA oxidation are variable in mice, consistently increased lipogenesis (Table 2) suggests a potential role for n-3 PUFA. On the contrary, CLA feeding increased n-3 PUFA content and decreased n-6 PUFA in the rats [119, 120] which could probably explain the differences in CLA effects between the two species. Although the exact mechanism of CLA action has not been elucidated, Banni et al. [121] has suggested that the metabolites of CLA, conjugated dienes (CD18:3, CD20:3, CD20:4), could compete with other PUFA at the level of formation and metabolism in liver and affect LC-PUFA synthesis.

3.3. CLA and SCD in Hepatic Lipid Metabolism. In the adipose, there are some similarities between the effects of trans-10, cis-12 CLA and the inhibition of SCD1. For example, reduced adiposity is observed with both dietary trans-10, cis-12 CLA and SCD1 inhibition and one could speculate that the effects of trans-10, cis-12 CLA are mediated through SCD1 as trans-10, cis-12 CLA decreases SCD1 in adipose [122]. However, a study with SCD1<sup>−/−</sup> mice showed that the antiobesity effects of trans-10, cis-12 CLA were independent of SCD1 gene expression and enzyme activity [123].

Unlike adipose, the effects of trans-10, cis-12 CLA are varied in liver (Table 2). While trans-10, cis-12 CLA decreased hepatic SCD activity in vitro [124], in vivo studies report increased hepatic SCD1 gene expression [32, 95]. In contrast to trans-10, cis-12 CLA effects in mice, SCD1<sup>−/−</sup> mice showed increased insulin sensitivity, reduced hepatic lipogenic genes, upregulated lipid oxidizing genes, increased hepatic saturated FA and unchanged hepatic n-3 and n-6 PUFA [125]. SCD1<sup>−/−</sup> mice fed trans-10, cis-12 CLA showed reduced hepatic accumulation compared to wild type [123] confirming that reduced SCD1 expression decreases hepatic lipid accumulation [126]. Liver specific SCD1 knock out decreased expression of SREBP1 and ChREBP and their target genes there by reducing hepatic lipogenesis [127]. In contrast, short-term inhibition of tissue specific hepatic SCD increased hepatic TG content and enhanced insulin signaling, [128] but the long-term inhibition decreased hepatic steatosis [129]. The differences in responses observed in liver specific knockout versus complete SCD knockout mice suggests that hepatic lipid metabolism is being affected by lipid metabolism in nonhepatic tissues [130].

As trans-10, cis-12 CLA effects in mice are mostly associated with insulin resistance; increased hepatic SCD1...
expression is probably due to increased SREBP-1c expression. Hepatic steatosis due to trans-10, cis-12 CLA is also seen in the absence of insulin and is associated with reduced expression of SCD1 and other lipogenic genes [33]. These results indicate that the disturbances in hepatic lipid metabolism caused by dietary trans-10, cis-12 CLA are mediated by multiple mechanisms [131] rather than through changes in SCD1 alone.

3.4. Role of Adipose during CLA-Induced Hepatic Steatosis. The effect of CLA on adipose lipid metabolism is well documented [14]. Of all the CLA isomers, trans-10, cis-12 CLA is the most potent to induce changes in adipose [25]. The changes may be caused by reduced lipid content, size, and number of adipocytes. Trans-10, cis-12 CLA reduces lipogenesis by decreasing expression of SREBP-1c and PPAR-γ and their downstream genes, ACC, FASN, and SCD1, reduces glucose and FA uptake by reducing GLUT4 and LPL expression, increases FA oxidation by increasing CPT1 and UCP2 expression and reduces adipocyte proliferation and differentiation by reducing PPAR-γ and its downstream genes [131]. Furthermore, CLA affects various adipocyte secreted-adipokines (e.g., leptin, adiponectin, and resistin) and cytokines (e.g., TNFa and IL6), which are involved in wide range of physiological activities [14]. Trans-10, cis-12 CLA increases the mRNA expression of cytokines, TNFa and IL6 in adipose tissue [131]. However, the circulating cytokine levels are reduced in response to trans-10, cis-12 CLA (Table 2). The increased cytokine expression in adipose tissue is known to reduce the activity of PPAR-γ [132], and thereby affect its downstream cellular functions. Also, TNFa and IL6 inhibit the activation of insulin receptor substrate-1 (IRS-1) through induction of suppressors of cytokine signaling (SOCS3) disrupting insulin action [133]. The expressions of TNFa and adiponectin, an adipokine associated with insulin sensitivity, are inversely related [134]. The adipose tissue depletion would reduce the level of adiponectin and when coupled with increased TNFa would lead to severe insulin resistance. The subsequent pancreatic β cell hyperplasia, as a compensatory mechanism to insulin resistance, leads to hyperinsulinemia which promotes lipid accretion in the liver leading to hepatic steatosis [39].

In mice, trans-10, cis-12 CLA causes severe lipodystrophy reducing the levels of leptin and adiponectin (Table 2), which leads to hepatic steatosis (Table 1, Figure 1). Re-establishing the levels of leptin or adiponectin either through external supplementation (in case of leptin) or induction using rosiglitazone (ROS) (in case of adiponectin) attenuated hepatic steatotic condition and normalized the insulin levels in CLA-fed mice [135, 136]. Similar results are seen in studies where prevention of lipodystrophy prevented lipid accumulation in the liver [135]. Serum insulin levels are directly correlated with liver TG, while serum adiponectin levels are inversely related [35]. Adipokines could improve the condition of the liver by lowering the insulin levels. However, hepatic steatosis is seen in mice even at low insulin levels [33], suggesting that different mechanisms could regulate the induction of hepatic steatosis depending on the animal’s physiological condition. The intensity of hepatic steatosis could be directly related to the relative amounts of adipose tissue. CLA-induced hyperinsulinemia and hepatic steatosis are observed only if there are corresponding decreases in the adipose tissue mass [40, 135]. Stout et al. [137], reported increases in diacylglycerol (DAG) concentration and membrane associated protein kinase C (PKC) during trans-10, cis-12 CLA-induced hepatic steatosis. Increased PKC would affect insulin signaling leading to insulin resistance, hyperinsulinemia, and hyperglycemia [137].

3.5. CLA and Inflammatory Responses. In addition to its effects on lipid metabolism, trans-10, cis-12 CLA also induces an inflammatory response in adipose tissue [131, 138]. Trans-10, cis-12 CLA activates integrated stress response leading to activation of NF-kB pathway, induction of inflammatory cytokines, TNFa, IL6, and IL8 [41, 138, 139], and macrophage infiltration [35]. However, the level of circulating cytokines, TNFa and IL6, were decreased in response to trans-10, cis-12 CLA [42, 43]. In contrast to the adipose, the effects of CLA on hepatic inflammatory responses are not well defined. Trans-10, cis-12 CLA did not affect expression markers of macrophage infiltration in mice liver such as TNFa or F4/80 and CD68 during hepatic steatosis [35]. However, trans-10, cis-12 CLA increased expression of markers of hepatic inflammation in hamsters without inducing hepatic steatosis [57]. The authors in [57] attributed this to an increased capacity of the liver for higher FA oxidation leading to inflammation and oxidant stress defense pathway in the hamsters.

4. Prevention or Amelioration of CLA-Induced Hepatic Steatosis

Several studies have examined either the prevention or amelioration of trans-10, cis-12 CLA-induced hepatic steatosis (Table 3) by normalizing serum adipokine levels, altering hepatic PUFA composition or both. External supplementation of recombinant murine leptin ameliorate CLA-induced hepatic steatosis and hyperinsulinemia by decreasing hepatic lipogenesis and increasing insulin sensitivity respectively [40, 136]. Serum adiponectin levels were not restored (and remained low) even after leptin supplementation, prompting the authors in [136] to claim that leptin alone could ameliorate CLA induced steatosis. Conversely, trans-10, cis-12 CLA caused hyperinsulinemia associated with lipid steatosis in Ob/Ob mouse which lack functional leptin [143] suggests the involvement of other factors. Increasing adiponectin levels by supplementation of ROSI attenuates liver fat accumulation in Ob/Ob mouse [49]. ROSI prevented lipodystrophy, decreased hepatic lipogenesis and subsequently liver TG content [35]. The insulin sensitizing action of leptin and adiponectin normalizes insulin levels which further helps in preventing CLA-induced steatosis [40, 141].

Dietary FA or oil supplements with higher n-3 and n-6 PUFA are able to ameliorate liver steatosis when supplemented along with CLA. Supplementing arachidonic acid [140] or its precursor γ-linolenic acid (18:3n-6) [44] decreased induction of hepatic steatosis and increased liver
### Table 3: Summary of literature studies on amelioration of CLA induced hepatic steatosis.

| Reference | No. per treatment | Study days | % Added dietary CLA | Treatment          | Treatment dose, %1 | Observations |
|-----------|-------------------|------------|---------------------|--------------------|--------------------|--------------|
| [136]     | 3 to 6            | 28         | 2.0                 | Leptin             | 5 μg/d             | ↓ Hepatic steatosis, ↑ insulin sensitivity, |
| [40]      | 5 to 14           | 30         | 1.0                 | Leptin             | 5 μg/d             | ↑ insulin sensitivity, ameliorated hepatic steatosis |
| [49]      | 5                 | 28         | 1.5                 | Rosiglitazone      | 10 mg/kg BW        | ↑ Insulin sensitivity, prevented depletion of epididymal adipose tissue |
| [35]      | 10                | 42         | 2.0                 | Rosiglitazone      | 10 mg/kg BW        | ↓ Hepatic TG content, ↓ hepatic lipogenesis, ↑ serum leptin and adiponectin, prevents lipodystrophy |
| [140]     | 7                 | 28         | 3.0                 | Arachidonic acid   | 1, 2               | ↓ Induction of hepatic steatosis, ↑ liver PGE2, ↑ epididymal adipose |
| [44]      | 7                 | 28         | —                   | γ-Linolenic acid   | 5                  | ↓ Hepatic steatosis, ↑ PGE2 |
| [38]      | 10                | 56         | —                   | Flax seed oil (α-Linolenic acid) | 0.39 | ↓ Steatosis, ↑ n-3 and n-6 PUFA in liver |
| [27]      | 7 to 8            | 22         | 1.0                 | Fish oil           | 1.5, 3, 6          | ↑ Leptin and Adiponectin, ↑ Insulin, ↑ TG in liver, ↓ fat pad |
| [141]     | 10                | 105        | 1.0                 | Pine oil           | 7.5                | Serum insulin levels stabilized over 3 weeks |
| [135]     | 5 to 6            | 100        | 1.0                 | 34% dietary fat    | 34% dietary fat    | Normal plasma insulin levels, ↑ liver weight |
| [45]      | 6                 | 28         | 2.0                 | DHA                | 0.5                | ↑ Fatty liver, ↑ FA synthesis, plasma leptin, and adiponectin unaffected |
| [142]     | 10                | 56         | —                   | DHA, EPA           | 0.5, 0.5           | Prevented hepatic steatosis, partially restored plasma leptin, only DHA restored plasma adiponectin |

1Percentage in the diet except wherever noted.

PGE2 levels. Hepatic steatosis is characterized by significant reduction in the levels of arachidonic acid in liver. Arachidonic acid supplementation would not only normalize the level of respective FA but would also increase the levels of hepatic PGE2 [44, 140]. Both arachidonic acid and PGE2 would further reduce hepatic lipogenesis by decreasing FASN and S14 gene expression [140, 144] thereby preventing hepatic steatosis.

The importance of n-3 PUFA concentrations on hepatic lipid metabolism was explained in the earlier section. Trans-10, cis-12 CLA decreases liver n-3 PUFA concentrations which affect hepatic lipid metabolism. Dietary supplements enriched in n-3 PUFA along with CLA diet increased the content of n-3 and n-6 PUFA in liver [38]. Fish oil, a source of PUFA has been shown to ameliorate CLA-induced steatosis by increasing leptin and adiponectin levels and decreasing plasma insulin [27]. Pinolenic oil, a source of Pinolenic acid was able to stabilize insulin levels when fed with CLA [141]. Similarly, flaxseed oil, a source of α-linolenic acid was able to increase n-3 and n-6 PUFA in liver. Supplementing EPA and DHA prevents lipid accumulation when fed with trans-10, cis-12 CLA [45, 142]. This effect was independent of their effects on stabilizing insulin sensitivity. Both EPA and DHA have modest effects in restoring plasma leptin levels, while DHA alone can restore plasma adiponectin levels to some extent [142]. The effects of DHA in preventing hepatic steatosis were mediated through decreasing hepatic lipogenesis [45].

### 5. Role of cis-9, trans-11 CLA in Hepatic Metabolism

Of the 16 naturally occurring CLA isomers, trans-10, cis-12 CLA and cis-9, trans-11 CLA have been the most extensively studied with respect to their bioactive properties. Most
of the animal studies have used a CLA mixture having trans-10, cis-12 CLA and cis-9, trans-11 CLA in 1:1 ratio to study the effect of CLA on liver metabolism. Studies using purified CLA isomer have delineated the differences between the two isomers. While trans-10, cis-12 CLA leads to decreased adipose tissue leading to insulin resistance, hyperinsulinemia, and hepatic steatosis, cis-9, trans-11 CLA shows only modest effects in mice [30–32, 86] and hamsters [56, 58]. Similarly, the effects of CLA on SCD1 gene and protein expression are isomer specific [145]. Contrary to trans-10, cis-12 CLA, cis-9, trans-11 CLA has no effect on SCD1 gene expression either in vitro [124] or in vivo [95].

A few studies have reported beneficial effects of cis-9, trans-11 CLA. For example, cis-9, trans-11 CLA did not alter liver lipid content but reduced 18:1n-9 and 18:1n-7 and increased 18:2n-6 in TG in contrast to trans-10, cis-12 CLA [108]. In addition, cis-9, trans-11 CLA promotes insulin sensitivity [42, 43] by reducing adipose inflammation [41, 132]. Furthermore, it enhances hepatic mitochondrial function and protects against oxidative stress by increasing activities of mitochondrial antioxidant enzymes [146]. The anti-inflammatory role of cis-9, trans-11 CLA is related to the induction of anti-inflammatory heat shock protein (HSP) 70 kDa and decreased expression of proinflammatory macrophage migration inhibitory factor [147].

6. Conclusions

Hepatic steatosis induced by trans-10, cis-12 CLA is associated with lipodystrophy in addition to insulin resistance, hyperinsulinemia, and hyperglycemia in mice (Figure 1). These effects are largely attributed to decreased adipokine (leptin and adiponectin) secretion. Dietary interventions preventing lipodystrophy or normalizing leptin and adiponectin levels prevents or ameliorates hepatic steatosis in mice, suggesting that adipose tissue responsiveness to trans-10, cis-12 CLA could be the main contributing factor. The moderate responsiveness of adipose tissue to trans-10, cis-12 CLA observed in hamsters and rats results in lower (or absence of) hepatic TG accumulation when compared with mice (Table 1) explains species specific responses.

Hepatic steatosis, due to increased lipid accumulation, is multifactorial and is largely attributed to increased rates of lipid synthesis along with lipid uptake, and it far exceeds the rates of FA oxidation and VLDL secretion. In addition, trans-10, cis-12 CLA-induced hepatic steatosis is characterized by reduction of n-6 PUFA (especially C20:4n-6) and n-3 PUFA (Figure 1). Changes in hepatic FA composition could play an important role in progression of hepatic steatosis, as normalizing the levels of n-6 PUFA or n-3 PUFA by dietary supplementation prevents or ameliorates hepatic lipid accumulation. Further studies are needed to understand the molecular mechanisms and the interrelationship between trans-10, cis-12 CLA-induced hepatic steatosis and altered hepatic PUFA content. We are still lacking mechanistic details showing relationship between adipokine levels, insulin resistance, and hepatic FA composition in context of hepatic steatosis, and it needs to be addressed in the future experiments.

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Research Article

Effects of Exercise Training on Molecular Markers of Lipogenesis and Lipid Partitioning in Fructose-Induced Liver Fat Accumulation

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The present study was designed to investigate the impact of exercise training on lipogenic gene expression in liver and lipid partitioning following the ingestion of a high fructose load. Female rats were exercise-trained for 8 wk or kept sedentary before being submitted to a fasting/refeeding protocol. Rats were further subdivided as follow: rats were fasted for 24 h, refed a standard diet for 24 h, starved for another 24 h, and refed with a standard or a high-fructose diet 24 h before sacrifice. Fructose refeeding was associated with an increase in hepatic lipid content, endocannabinoid receptor 1, sterol regulatory element-binding protein1c, and stearoyl-CoA desaturase1 gene expression in both Sed and TR rats. However, desaturation indexes measured in liver (C16 : 1/C16 : 0 and C18 : 1/C18 : 0) and plasma (C18 : 1/C18 : 0) were higher (P < 0.01) in TR than in Sed rats following fructose refeeding. It is concluded that exercise training does not significantly affect fat accumulation and the molecular expression of genes involved in lipogenesis after fasting and fructose refeeding but does modify the partitioning of lipids so as to provide more unsaturated fatty acids in liver without affecting liver fat content.

1. Introduction

The recent interest in liver fat metabolism has been spurred by the finding that obesity in Western societies results in an accumulation of hepatic lipids that, in turn, is associated with the deterioration of insulin signalling [1–3]. The clinical relevance of lipid handling by the liver has been enlightened by the finding that, when rats are starved and subsequently refed, liver lipids rapidly accumulate, especially when fed carbohydrates [4]. This phenomenon is closely associated to repeated bouts of weight loss and regain, also known as weight cycling or “yo-yo” dieting [5]. At the gene expression level, a food deprivation/refeeding regimen using high-carbohydrate diets results in the upregulation of several hepatic lipogenic enzymes such as fatty acid synthetase (FAS) [6–8] and stearoyl-CoA desaturase1 (SCD1) [9, 10]. Although no study has reported the effects of fasting/refeeding high-carbohydrate diets on endocannabinoid receptor 1 and 2 (CB1-2), experimental evidence suggests that hepatic CB receptors contribute to the development of diet-induced hepatic steatosis through the induction of the lipogenic transcription factor SREBP1c and its target enzymes (i.e., FAS) [11, 12]. Simple sugars, abundantly found in fruit drinks, sports drinks, and soda, seem to strongly induce obesity and hepatic steatosis in comparison to complex carbohydrates [13–15]. Among simple sugars, fructose is the most potently lipogenic inducer that can increase lipogenesis through activation of SREBP1c dependent and independent mechanisms [14, 16].

On the other hand, chronic exercise training affects liver fat metabolism by reducing the accumulation of liver lipids in high-fat fed animals and in humans [17–19]. Although
these training adaptations would be compatible with a decrease in long-term fat accumulation, it may represent a disadvantage in an acute situation, such as food deprivation/refeeding a high-fructose diet, in which an increase in hepatic lipogenic activity is necessary to rapidly and adequately buffer the large arrival of substrates. Investigating the role of training on the short-term management of fructose by the liver, in a food-deprived/refed situation, has been limited to the impact of an acute bout of exercise that inhibits key hepatic lipogenic enzymes [7, 8]. Proper handling of substrates by the liver may have subsequent implications on plasma TAG clearance and fat storage, resulting in a preferential accumulation of palmitate (C16:0) in muscle fat [22]. Physical inactivity also decreases dietary palmitate (C16:0) but not oleate (C18:1) oxidation, suggesting that the desaturation status of fatty acids is an important factor in determining their fate.

The first purpose of the present study was, therefore, to determine the effects of an 8-week-exercise training program on HFr refeeding-induced lipogenesis by measuring the expression of the lipogenic genes CB1, CB2, SREBP1c, SCD1, and fatty acid amide hydrolase (FAAH), an endocannabinoid degrading enzyme known to be involved in liver lipid infiltration [11, 23, 24]. It is postulated that under the present fasting/refeeding protocol, TR would be associated with an increased hepatic lipogenic activity. The second objective was to examine the effects of TR on lipid partitioning by measuring fatty acid desaturation index of SCD1 activity in liver and plasma expressed under the ratio of monounsaturated fatty acid desaturation status of fatty acids is an important factor in determining their fate.

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2. Materials and Methods

2.1. Animal Care. Female Sprague-Dawley rats (Charles River, St-Constant, PQ, Canada), weighing 180–200 g (6 weeks of age), upon their arrival were housed individually and had ad libitum access to food and tap water (n = 40). Female rats were used in the present study to avoid the decrease in food intake and body weight that was observed in exercise-trained male rats [25, 26]. Their environment was controlled in terms of light (12:12-h light-dark cycle starting at 6:00 AM), humidity, and room temperature (20–23°C). This experiment was conducted according to the directives of the Canadian Council on Animal Care after the University of Montreal (Montreal, PQ, Canada) approval.

2.2. Exercise Training Protocol. Four days after their arrival, all animals were randomly assigned to a sedentary (Sed) or an exercise-trained group (TR). Exercise training consisted of continuous running on a motor-driven rodent treadmill (Quinton Instruments, Seattle, WA) 5 times/week for 8 weeks. Exercise intensity was progressively increased from 15 min/day at 15 m/min, 0% slope, up to 60 min/day at 26 m/min, 10% slope, for the last 4 weeks of the program. Based on previous measurements of oxygen consumption during a progressive exercise test in rats, it was estimated that exercise intensity during the last 4 weeks of the training program occurred at ~65% of maximal oxygen consumption [27]. At the end of this 8-week period, animals were sacrificed 36 to 48 h after the last exercise session.

2.3. Dietary Treatment Protocol. During the first complete 7 weeks, Sed and TR animals had free access to a standard diet (SD; 12.5% lipid, 63.2% carbohydrate, and 24.3% protein; kcal) consisting of usual pellet rat chow (Agribands Canada, Woodstock, ON). Toward the end of the 8th week that corresponded to 4 days prior to sacrifice, animals were submitted to two fasting and refeeding cycles [10]. Sed and TR animals fasted for 24 h, refed the SD diet for 24 h, starved for another 24 h, and then refed either the SD or an isoenergetic high-fructose (HFr) diet for 24 h. The HFr diet consisted of 13% lipid, 66.8% carbohydrate mainly fructose, and 20.2% protein (kcal). Details of the diets are presented in Table 1. Body weight and food intake were monitored 3 times/week in all rats during the first 7 weeks and every day during the fasting/refeeding stage.

2.4. Blood and Tissue Samplings. All animals were sacrificed between 09:00 and 11:00 AM. Food was removed from the animals’ cage at least 3 h before sacrifice. After complete anaesthesia (pentobarbital sodium, 50 mg/kg ip), the abdominal cavity was rapidly opened along the median line of the abdomen. Blood was rapidly (∼45 s) drawn from the abdominal vena cava (∼4 mL) into syringes pretreated with EDTA (15%). Thereafter, blood was centrifuged (3,000 rpm for 10 min, 4°C). The liver median lobe was freeze-clamped and used for mRNA, Western blot, and lipid analysis. The mesenteric (Mes), urogenital (Ug), and retroperitoneal (Rp) fat deposits were excised and weighed in that order. Mesenteric fat pad consisted of adipose tissue surrounding the gastrointestinal tract from the gastrointestinal sphincter to the end of the rectum with special care taken in distinguishing and removing pancreatic cells. Urogenital fat pad included adipose tissue surrounding the kidneys, ureters, and bladder in addition to the ovaries, oviducts, and uterus.
Table 2: Real-time PCR primer sequences.

| Gene     | Sense (5'-3') | Antisense (5'-3') | Accession no. |
|----------|---------------|-------------------|---------------|
| CB1      | AAGGACCTGAGACATGCTTCCGA | TCACCAGCGCTCTTTCCTGA | X55812        |
| CB2      | GGGGGCTGGATGTTGCTTCTA | ACGATGGTGGTGGCTTCCA | NM_020543     |
| FAAH     | AGCATGCCAGATGGACCTCTCA | GCATGAACTCAGACACAGCTT | U72497       |
| SCD1     | CCTAACCCTGAGATCCGGTCTA | AGCCCATAAAAGATTCTGCAA | J02585       |
| SREBP1c  | ACGACCGAACGCTGATGGACAC | CCGGAAAGCGGCTTGAAGTACC | L16995      |
| β-actin  | CATGAATGATCAAGATCATGCTCCT | CTGCTTGCATCCACATCG | V01217       |

CB1: endocannabinoid receptor1.
CB2: endocannabinoid receptor2.
FAAH: fatty acid amide hydrolase.
SCD1: stearoyl-CoA desaturase1.
SREBP1c: sterol regulatory element-binding protein1c.

Retroperitoneal fat pad was taken from the fat deposit behind each kidney along the lumbar muscles. Skeletal muscles (plantaris, soleus, medial, and lateral gastrocnemius) of the right limb were removed and weighed thereafter. All tissue samples were immediately frozen in liquid nitrogen after being weighed. All plasma and tissue samples were stored at −80°C until analyses.

2.5. Analytical Procedures

2.5.1. Quantitative Real-Time PCR. Total RNA was extracted from frozen liver tissue with the use of either TRIzol (Invitrogen Canada Inc, Burlington, ON) or PureLink RNA mini Kit (Invitrogen, Carlsbad, CA) according to the manufacturer’s protocol. RNA was treated with DNase (Invitrogen) in order to avoid genomic contamination. Treated RNA was reverse-transcribed into cDNA using either the random hexamer primers (Invitrogen) and reverse transcriptase (Invitrogen), or the transcriptor first-strand cDNA synthesis kit Roche Diagnostics, Mannheim, Germany. Subsequently, we added 2 μL cDNA to 18 μL of a reaction mixture containing SYBR Green Supermix from Bio-Rad Laboratories Inc. for SCD1 and SREBP1c and from Roche Diagnostics Manheim, Germany, for CB1, CB2, and FAAH. As for the previous steps, polymerase chain reaction (PCR) was performed in two different PCR machines. An iCycler IQ Real-Time PCR detection system (Bio-Rad Laboratories, Inc., Hercules, CA) was used for SCD1 and SREBP1c, and a Roche LightCycler 480 Instrument (Roche Diagnostics Manheim, Germany) served for CB1, CB2, and FAAH mRNA quantification. All samples were analysed in duplicate. The gene-specific primers were purchased from Invitrogen Life Technologies Inc. and are listed in Table 2. We optimized the PCR reaction protocol according to manufacturer’s recommendations. For SCD1 and SREBP1c, the thermal cycling program was 95°C for 2 min, followed by 40 cycles at 95°C for 25 s, at 60°C for 25 s, and at 72°C for 40 s. For the quantification of CB1, CB2, and FAAH mRNA, the following procedure was used. Preincubation lasted 10 min at 95°C, followed by 45 cycles of PCR at 95°C for 15 s, 60°C for 15 s, and 72°C for 15 s. Following PCR, the melting curve was completed to ensure that only one PCR product was amplified per reaction. The relative gene expression was calculated as a function of $2^{-\Delta\Delta Ct}$ and normalized for β-actin transcript level. The equation used in the calculation was as follows: fold induction = $2^{\Delta\Delta Ct}$, where Ct = the threshold cycle, (i.e., the cycle number at which the sample’s relative fluorescence rises above background fluorescence) and ΔΔCt = [Ct gene of interest (unknown sample) – Ct β-actin (unknown sample) – Ct gene of interest (calibrator sample) – Ct β-actin (calibrator sample)].

2.5.2. Western Blot Analysis. Liver tissue was prepared by homogenization in RIPA (Radioimmunoprecipitation Assay) buffer (50 mM Tris HCl pH 8, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulphate (SDS)) and 10−5 M protease inhibitors (phenylmethyl sulfonyl fluoride (PMSF), EDTA, and Pepstatin A) then centrifuged at 10,000 g for 20 min, at 4°C. The supernatants were collected, and protein concentrations were determined using Bradford assay. Then, 30 μg of total protein were applied to each well of 12% SDS polyacrylamide gel and electrophoresed at 4°C for 2 h at 130 V. The resolved protein bands were then transferred onto nitrocellulose membranes (Hybond-C Extra; Amersham Biosciences) at 30 V for 120 min, at room temperature. The blots were blocked overnight at 4°C with blocking buffer (10% nonfat milk in 10 mmol/L Tris, pH 7.5, 100 mmol/L NaCl, 0.1% Tween 20). The blots were incubated for 1 h at room temperature with primary antibody rabbit polyclonal anti-SCD1 (1 : 2000) kindly provided by Dr. Juris Ozols, then with horseradish peroxidase-labeled donkey antirabbit IgG (1 : 10,000 dilution, Amersham Biosciences UK) for 2 hours at room temperature as secondary antibody. Protein loading was normalized for β-actin (Sigma monoclonal anti-β-actin antibody produced in mouse at 1 : 5000 dilution as a primary antibody and horseradish peroxidase-conjugated IgG anti-mouse as secondary antibody). Finally the blots were visualized using the ECL (enhanced chemiluminescent, Amersham RPN 2132) and exposed to autoradiography film (Eugene, St-Laurent, QC). Density of the bands was performed with the use of Photoshop software.

2.5.3. Liver Tissue Preparation for Lipid Analysis Using the Folch Method [28]. Samples of 150–200 mg frozen liver tissue were homogenized in a saline buffer supplemented
Table 3: Body weight, energy intake, sum of intra-abdominal fat pad weights (mesenteric, urogenital, and retroperitoneal), sum of 4 muscle weights (soleus, plantaris, medial, and lateral gastrocnemius), plasma glucose, insulin, and leptin in sedentary (Sed) and trained (TR) rats submitted to a fasting/refeeding protocol. Animals were fed a standard (SD) diet during 7 wks and thereafter were starved for 24 h and fed a SD diet for 24 h and starved for another 24 h and then fed the SD or the high-fructose (HFr) diet 24 h prior to sacrifice at the end of the 8th week.

| Diets | SD diet | TR | Sed | HFr diet | TR |
|-------|---------|----|-----|----------|----|
| Body weight (g) | 281 ± 8 | 292 ± 8 | 287 ± 9 | 291 ± 7 |
| Energy intake (kcal/last 24 h) | 84.7 ± 2.9 | 88.2 ± 4.2 | 57.9 ± 4.5 | 49.4 ± 4.9 |
| Sum of intra-abdominal fat pad weights (g) | 16.12 ± 1.9 | 15.2 ± 1.6 | (P = 0.058) | 21.3 ± 2.3 | 14.8 ± 1.56 | (P = 0.058) |
| Sum of 4 muscle weights (g) | 1.96 ± 0.05 | 2.08 ± 0.05 | 1.97 ± 0.04 | 2.07 ± 0.05 |
| Plasma glucose (mmol/L) | 7.7 ± 0.3 | 8.1 ± 0.3 | 8.1 ± 0.4 | 7.3 ± 0.2 |
| Plasma insulin (pM) | 456 ± 67 | 573 ± 66 | 437 ± 65 | 447 ± 59 |
| Plasma leptin (ng/mL) | 2.62 ± 0.4 | 2.26 ± 0.2 | 3 ± 0.4 | 2.02 ± 0.2 |

Values are means ± SE, n = 10 rats/group.

*Significantly different from corresponding values under the SD diet, P < 0.01. †Significantly different from Sed rats under the same diet condition, P < 0.05.

with 1% antiprotease (PMSF, Pepstatin, leupeptin) and 1% antioxidant (butylated hydroxytoluene, BHT). Lipid extraction from liver tissue was performed overnight at 4°C with the use of a 2:1 chloroform-methanol mixture (vol/vol). Then, saline was added and the lower phase was removed into glass vials and dried under a stream of nitrogen [29]. Tubes were immediately stored at −80°C for further fatty acid measurements.

2.5.4. Plasma and Liver Fatty Acid Composition Analysis and SCD1 Desaturation Index. The method used for fatty acid composition analysis was slightly adapted from the technique described by Lepage and Roy [30]. Plasma (100 μL) or prepared liver tissue (described above) was used for this analysis. The samples were dissolved in 2 mL methanol-hexane 4:1 (v/v) mixture containing BHT and an internal fatty acid standard (Nonadecanoic acid C19:1; NU-Check Prep. Inc., Elysian, MN, USA). Then, 200 μL of acetylchloride was added to each tube. Samples were hydrolysed at 100°C for 60 min, followed by the addition of 5 mL of 6% K2CO3. The upper phase was extracted and then analysed by gas chromatography. As described by Mainieri et al. [29] using the quantitative values of palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), and oleic acid (C18:1), the desaturation index of SCD1 was determined using the product/substrate ratio (C16:1/C16:0 and C18:1/C18:0).

2.5.5. Liver TAG. Liver TAG concentration was estimated from glycerol released after ethanolic KOH hydrolysis by a colorimetric method using commercial kits from Sigma (St. Louis, Missouri, USA). Although this method does not discriminate between glycerol from phospholipids or TAG, Frayn and Maycock [31] have shown that omitting removal of phospholipids leads to only a ± 2% error in the determination of muscle TAG. Thus, although a small amount of free glycerol may be produced from hydrolysis of phospholipids, it is considered negligible.

2.5.6. Plasma FFA and TAG. Plasma total free fatty acid (FFA) concentrations were measured by a colorimetric assay with commercially available kits from Roche Diagnostics (Penzberg, Germany). Plasma TAG was measured using a colorimetric method (Sigma; St. Louis, Missouri, USA).

2.5.7. Plasma Glucose, Insulin, and Leptin. Plasma glucose concentrations were determined with the use of a glucose analyser Yellow Springs Instruments 2300 (Yellow Springs, Ohio, USA). Plasma insulin and leptin concentrations were measured with commercially available radioimmunoassay kits (Linco Research, St-Charles, Missouri, USA).

2.6. Statistical Analysis. Values are expressed as means ± S.E. Statistical analyses were performed with the use of a two-way ANOVA for nonrepeated measures using training status and diet as main effects. Fisher LSD post hoc test was applied where appropriate, and P values < 0.05 were considered significant.

3. Results

Feeding rats an HFr diet after starvation failed to induce changes in body weight, abdominal adiposity, muscle weight, circulating glucose, insulin, and leptin, even though it decreased energy intake (P < 0.01) (Table 3). Exercise training, however, was associated with a significant increase in energy intake as well as in the sum of 4 muscle weights and with a decrease in intra-abdominal adiposity and in plasma leptin levels under the two diet conditions (P < 0.05).

HFr compared to SD refeeding resulted in higher (P < 0.05) gene expression of SCD1, SREBP1c, and CB1 along with protein content of SCD1 (Figure 1). Training, however, had no effect on the expression of any of these molecular markers.

As expected, HFr refeeding resulted in higher liver and plasma TAG as well as plasma FFA levels (Figure 2). Although liver TAG levels were not affected by training, plasma TAG
**Figure 1:** Gene expression of hepatic stearoyl-CoA desaturase1 (SCD1), sterol regulatory element-binding protein1c (SREBP1c), endocannabinoid receptors (CB1 and CB2), fatty acid amide hydrolase (FAAH), and protein content of liver SCD1 relative to β-actin in sedentary (Sed) and trained (TR) rats submitted to a fast/refeeding protocol. Animals were fed a standard (SD) diet during 7 wks and thereafter were starved for 24 h and fed with the SD diet for 24 h and starved for another 24 h and then fed with the SD or the high-fructose (HFr) diet 24 h prior to sacrifice at the end of the 8th week. Values are means ± SE with n = 10 rats/group except for SCD1 protein level (n = 4–6 rats/group). &: significantly different from corresponding values under the SD diet, P < 0.05, &&: P < 0.01.
Figure 2: Liver and plasma triacylglycerol (TAG) and plasma free fatty acid (FFA) in sedentary (Sed) and trained (TR) rats submitted to a fast/refeeding protocol. Animals were fed a standard (SD) diet during 7 wks and thereafter were starved for 24 h and fed with the SD diet for 24 h and starved for another 24 h and then fed with the SD or the high-fructose (HFr) diet 24 h prior to sacrifice at the end of the 8th week. Values are means ± SE with n = 10 rats/group. &: significantly different from corresponding values under the SD diet, P < 0.05, &&: P < 0.01. +: significantly different from Sed rats under the same diet condition, P < 0.05, ++: P < 0.01.

4. Discussion

In an attempt to further understand the role played by regular exercise training on liver fat metabolism and accumulation, we designed the present study to determine if an exercise training program may affect the acute handling of a fructose load by the liver. Using a fasting/HFr refeeding approach, we found that gene expression of hepatic molecular markers of the lipogenesis pathway and as well liver fat accumulation were highly but similarly increased by the HFr refeeding in Sed as well as in TR rats. To go one step further, we measured the ratio of monounsaturated to saturated fatty acids, which is used as a surrogate marker for SCD1 activity in liver, thus providing a physiological assessment of the fructose disposal by the liver and fatty acid partitioning [14, 32]. We report for the first time that, following the ingestion of a fructose load, the SCD1 desaturation indexes were higher in liver (C16:1/C16:0 and C18:1/C18:0) and in plasma (C18:1/C18:0) of TR compared to Sed animals. This finding and FFA levels were, respectively, higher (P < 0.05) and lower (P < 0.01) in TR animals refed with the HFr diet.

SCD1 fatty acid desaturation indexes (C16:1/C16:0 and C18:1/C18:0) in the liver were significantly (P < 0.01) increased exclusively in TR animals refed the HFr diet as a result of a synergetic effect of TR and HFr refeeding (Figures 3(a) and 3(b)). In plasma, HFr refeeding induced an increase in C16:1/C16:0 in both Sed and TR rats and an increase in C18:1/C18:0 only in TR rats (Figures 3(c) and 3(d)).
Figure 3: Stearoyl-CoA desaturase1 (SCD1) fatty acid desaturation indexes expressed under the form of the ratio of monounsaturated (C16 : 1, C18 : 1) over saturated (C16 : 0, C18 : 0) fatty acids measured in liver and plasma of sedentary (Sed) and trained (TR) rats submitted to a fast/refeeding protocol. Animals were fed a standard (SD) diet during 7 wks and thereafter were starved for 24 h and fed with the SD diet for 24 h and starved for another 24 h and then fed with the SD or the high-fructose (HFr) diet 24 h prior to sacrifice at the end of the 8th week. Values are means ± SE, n = 10 rats/group for plasma and n = 5 rats/group for liver desaturation indexes. &&: significantly different from corresponding values under the SD diet, P < 0.01. ++: significantly different from Sed rats under the same diet condition, P < 0.01.

indicates that training affects the acute handling of a fructose load by providing a higher rate of conversion of de novo synthesized saturated fatty acids to the monounsaturated form.

The HFr refeeding in Sed animals did not affect the fatty acids desaturation indexes measured in the liver (Figures 3(a) and 3(b)). This indicates that the HFr diet did not result in a change in the ratio of saturated versus monounsaturated fatty acids accumulated in the liver. However, the C16 : 1/C16 : 0 desaturation index of Sed rats refed with the HFr was highly increased in plasma, suggesting an increased liver exportation of the fatty acids under the desaturated form. Several studies have demonstrated that fatty acids originating from different sources appear to be managed differently in the liver with regard to their use for storage or secretion [33, 34]. More specifically, it seems that newly synthesized fatty acids are channelled preferentially into very-low-density lipoproteins for exportation [35]. Plasma TAG and FFA plasma levels were accordingly increased by the HFr refeeding in Sed rats. However a somewhat different picture is found in TR rats. First, liver SCD-1 desaturation indexes were highly increased following training without any effects on liver TAG levels. This indicates that although the same quantity of fat was accumulating in liver of Sed and TR animals after the HFr refeeding, more of the fatty acids stored in liver of TR rats were under the desaturated form.
This as such provides new evidence that training affect, the acute handling of a fructose load. It might be argued that the increased desaturation ratio in liver of TR rats might be due to a decreased exportation of desaturated fatty acids. This possibility, however, is not supported by the finding that the C16:1/C16:0 desaturation ratio in plasma was similar between Sed and TR rats and the C18:1/C18:0 ratio was higher in TR than in Sed animals. The present data, therefore, support the interpretation that exercise training may influence the acute management of a substrate load such as fructose by changing the partitioning of newly synthesized fatty acids. Supporting this view is the recent report that physical inactivity favours the accumulation of palmitate (C16:0) in muscle fat and decreases dietary palmitate but not oleate (C18:1) oxidation thus leading to the deterioration of insulin signalling [22].

The higher levels of fatty acids under the desaturated form in liver of TR rats refed with the HFr load may present some advantages. Recently, Li et al. [36] reported that SCD1 plays a key role in prevention of steatohepatitis by partitioning excess lipid into monounsaturated fatty acids (MUFAs) that can be safely stored. This indicates that training favouring the formation of fatty acids that are mostly unsaturated in liver could represent a molecular mechanism of exercise-training-induced metabolic protection in liver. Newly synthesized MUFAs C16:1 and C18:1 are the most abundant fatty acids founds in TAG molecules [37]. In this regard, exercise training might favour the conversion of the de novo synthesized fatty acids that are cytotoxic molecules into a form more easily transferrable to TAG, known to be biologically inert molecules [38, 39].

Exercise training did not, under the present acute dietary manipulations, result in lower levels of liver fat accumulation. The effects of exercise training in reducing liver fat accumulation under long-term high-fat diets have been reported in several studies [17, 40]. The absence of effects of exercise training on liver fat accumulation in the present study is most likely due to the powerful effect of the HFr load on lipogenesis. HFr refeeding, whether in Sed or TR rats, was associated with an increase in hepatic gene expression of SCD1, SREBP1c, and the endocannabinoids CB1 receptors. It has been reported that the activation of CB1 receptors in liver stimulates de novo lipogenesis through induction of SREBP1c and its target enzymes [12]. Recent data in liver-specific CB1 knockout mice indicate that hepatic CB1 receptors are required for the development of diet-induced steatosis by increasing de novo lipogenesis and inhibiting fatty acid oxidation [11]. In agreement with this statement, our data show, for the first time, that gene expression of CB1, and to a lesser extent CB2, was increased in liver of fasted and fructose refed animals in comparison to those refed the SD diet. Additionally, it has been reported that lipid accumulation in liver may also occur by reduced expression and/or activity of fatty acid amidase hydrolase (FAAH), one of the endocannabinoid degrading enzymes [24]. The present results, however, do not indicate any effects of the high-fructose diet on the gene expression of the FAAH enzyme.

The fact that SCD1 gene expression and protein content in liver were not changed in TR compared to Sed rats under the HFr refeeding might at first glance contradict the finding of higher SCD1 desaturation indexes measured in the same rats. As stated above, the SCD1 index reflects the activity of SCD1. It is possible that since the HFr load highly stimulated the lipogenesis pathway that the discrimination between the TR and the Sed state might be possible only at the activity level.

An intriguing observation that needs to be addressed is the fact that several hours after the HFr refeeding, TR rats had higher plasma TAG and lower plasma FFA levels than their Sed counterparts. The present study provides no information on VLDL secretion rate. However, since liver TAG levels were similar in TR and Sed rats, it is unlikely that a difference in VLDL synthesis and/or secretion rate is responsible for the higher plasma TAG levels found in TR animals. In search of a different explanation, we must first acknowledge the fact that energy intake was ~30% higher in TR than in Sed rats refed the HFr diet. On a speculative basis, it is possible that the lower plasma TAG levels found in Sed compared to TR rats might be linked to a greater ability of Sed rats to store fat in peripheral adipose tissue under the present acute nutritional manipulations. Although the inter-diet comparisons did not reach the statistically significant level, the HFr compared to the SD refeeding seems to have resulted in higher intra-abdominal fat accumulation in Sed (from ~16 to 21 g) than TR rats (from 16 to 15.8 g; Table 3). In connection with this, higher plasma FFA levels measured in Sed compared to TR rats refed the HFr could result from a higher rate of basal lipolysis due to the higher adipocyte fat accumulation [41].

In summary, exercise training is associated with higher liver (C16:1/C16:0 and C18:1/C18:0) SCD1 desaturation indexes in rats submitted to a 2-day fast/refeeding protocol using a high-fructose diet in the second day. Gene expression of lipogenic molecular markers including hepatic endocannabinoid receptors, transcription factor SREBP1c, and SCD1 protein content was increased to a similar in extent in Sed and TR rats refed with the HFr diet. It is concluded that the pattern of management of the HFr load in a fast/refeed protocol is modified in exercise trained animals so as to provide more fatty acids under the unsaturated form in liver and plasma. These data support the contention that exercise training positively modifies the handling of an acute substrate load.

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Research Article

Exercise and Omega-3 Polyunsaturated Fatty Acid Supplementation for the Treatment of Hepatic Steatosis in Hyperphagic OLETF Rats

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1. Introduction

It is estimated that 20–40% of the general population and 50–90% of obese people have nonalcoholic fatty liver disease (NAFLD) [1, 2]. The presence of NAFLD has been described as an independent risk factor for the development of cardiovascular disease [3–5], and it often accompanies other obesity-related diseases such as type 2 diabetes and is considered the hepatic manifestation of the metabolic syndrome [6]. Further, NAFLD is associated with increases in all-cause and liver-related mortality [7, 8]. As a result, the examination of affordable treatment options for NAFLD, such as exercise or omega-3 polyunsaturated fatty acid (n3PUFA) supplementation, is becoming more imperative. Unfortunately, there is a paucity of studies investigating exercise and exercise combined with n3PUFA supplementation as treatments for NAFLD. Most exercise research has been focused on prevention, and not on treatment or reversal of preexisting NAFLD.
We have previously used the Otsuka Long-Evans Tokushima Fatty (OLETF) rats to investigate NAFLD because they spontaneously develop obesity, insulin resistance, and NAFLD which progressively worsens with advancing age [9, 10]. OLETF rats lack the cholecystokinin-1 receptor resulting in hyperphagia due to defective satiety signaling [11]. Further, a novelty of this particular model is their intrinsic aptitude for exercise (EX) on voluntary running wheels, a characteristic that is uncommon in obese rodent models. We have previously shown that EX on voluntary wheel running beginning at 4 weeks of age in OLETF rats prevents the development of hepatic steatosis, effects that were associated with prevention of insulin resistance and increased hepatic fatty acid oxidation (FAO) [9]. Moreover, we have established that locking of the wheels and ceasing daily EX in OLETF rats quickly activate a subgroup of precursors and processes known to initiate hepatic steatosis, including decreased hepatic mitochondrial oxidative capacity, increased hepatic expression of de novo lipogenesis proteins, and increased hepatic malonyl CoA levels [12]. Other labs have also shown that exercise can prevent fatty liver in rodents fed a high-fat-diet to induce obesity [13, 14]. Although we and others have established that daily exercise is an invaluable intervention to prevent NAFLD, it remains unknown if exercise is an effective treatment strategy for preexisting NAFLD.

Dietary polyunsaturated fatty acids (n3PUFAs) have also been studied as a treatment for NAFLD. n3PUFAs both activate a transcription factor that is important for hepatic oxidative capacity (peroxisome proliferator-activated receptor (PPARα)) and inactivates a master regulator of hepatic lipid synthesis (sterol regulatory element binding protein (SREBP)-1c) in rodents [15–22] and humans [23, 24]. This dual action on hepatic metabolism suggests that n3PUFAs could lower hepatic fat content. In OLETF rats, dietary intake of n3PUFAs beginning at 5 weeks of age prevented hepatic steatosis by decreasing the activity of lipogenic enzymes such as fatty acid synthase (FAS) and stearoyl CoA desaturase (SCD)-1 and decreasing mRNA expression of several key lipogenic genes including SREBP-1c and acetyl CoA carboxylase (ACC) [25]. The authors also found that mRNA expression and activity of CPT, a protein that is controlled by PPARα, were increased in the liver of n3PUFA-fed OLETFs [25]. These results indicate that n3PUFAs were effective at preventing increased liver TAG accumulation in OLETF rats; however, this study also addressed prevention and not treatment of NAFLD.

Treatment of NAFLD with n3PUFAs has also been examined previously. Seven days of dietary consumption of n3PUFAs led to reductions, but not complete reversal, of hepatic steatosis and systemic insulin resistance in ob/ob mice, associated with the down-regulation of SREBP-1c [19]. The authors also observed increased activation of PPARα and its associated gene targets suggesting increased β-oxidation in the liver, but lacked a quantitative measure of FAO. In another study, dietary intake of n3PUFAs attenuated, but was again not able to completely reverse, liver TAG content following high-fat-diet-induced hepatic steatosis [20].

To our knowledge, we are unaware of any studies that have examined the individual and combined effects of exercise and n3PUFA supplementation on hepatic mitochondrial FAO and treatment of NAFLD. Thus, the purpose of this study was to examine the individual and combined effects of exercise and the dietary intake of omega-3 fatty acids (3% of energy from fish oil) to treat preexisting hepatic steatosis in OLETF rats. We also examined the effects of these treatments on factors known to play a role in the development of hepatic steatosis including peripheral insulin sensitivity, hepatic FAO, and markers of hepatic lipogenesis. We hypothesized that both exercise and n3PUFAs would reverse or attenuate progression of NAFLD in obese rats and that the combination of treatments would have an even greater effect on treating NAFLD.

2. Methods

2.1. Study Design. The animal protocol was approved by the Institutional Animal Care and Use Committee at the University of Missouri. Male OLETF rats were generously provided by the Tokushima Research Institute, Otsuka Pharmaceutical (Tokushima, Japan) and arrived at 4 weeks of age. Rats were given ad libitum access to normal chow food (Purina Formulab Diet, 5008) and water in a temperature- and light-controlled room (12 h light-12 h dark cycle). At 13 weeks of age, an age when obesity, insulin resistance, and NAFLD are present in sedentary OLETF rats [10], OLETFs were randomly divided into 4 groups (n = 8/group): (1) remained sedentary (SED) on normal chow, (2) was provided access to voluntary running wheels (EX) with normal chow, (3) was provided diet supplemented with 3% of energy from fish oil (n3PUFA-SED), and (4) had access to running wheels and diet supplemented with 3% of energy from fish oil (n3PUFA-EX). Thus, treatments began at 13 weeks of age and ended at 20 weeks of age when rats were euthanized. Food consumption and body weights were assessed weekly, and running distances (wheels outfitted with a Sigma Sport BC 800 bicycle computer (Cherry Creek Cyclery, Foster Falls, VA, USA) for measuring daily running activity) in EX and n3PUFA-EX rats were measured daily following the end of the dark cycle during the 8-week treatment period. After being fasted and having wheels locked for a 5 h period, rats were anesthetized with sodium pentobarbital (100 mg/kg), tissue and blood were collected, fat pads were weighed, and rats were euthanized by exsanguination.

2.2. Diet. The normal chow diet used was Formulab 5008 (Purina Mills, St. Louis, Mo, USA) which we have used in previous studies in which the OLETF rats develop steatosis [9, 10]. The n3PUFA diet was custom-formulated at the University of Missouri using an AIN-93G diet supplemented with 3% of energy from menhaden fish oil. The menhaden fish oil was kindly donated by Omega Protein, Inc. (Houston, Tex, USA) and was stabilized with a synthetic antioxidant (0.2 g/kg tertiary butyl-hydroquinone (TBHQ)) and 1000 mg/kg mixed tocopherols to protect it from autooxidation. The percent of energy provided by fat
was the same between the Formulab 5008 and n3PUFA diet, 16.7%, respectively.

2.3. Intraperitoneal Glucose Tolerance Tests. Baseline glucose values were determined following a 12 h overnight fast one week prior to sacrifice (19 weeks of age). Glucose (2.0 g/kg) was then administered intraperitoneally, and tail blood draws were taken prior to and at 15, 30, 45, 60, and 120 min following the bolus of glucose as performed previously by our group [26].

2.4. Dual-Energy X-Ray Absorptiometry (DEXA). Whole body composition was determined using a Hologic QDR-1000/w DEXA machine calibrated for rats as performed previously by our group [27].

2.5. Serum Measures. The concentrations of glucose (Sigma Aldrich, St. Louis, Mo, USA), insulin (Millipore, Billerica, Mass, USA), β-hydroxybutyrate (Stanbio Laboratory, Boerne, Tex, USA), triglycerides (Sigma Aldrich), and free fatty acids (Wako, Richmond, Va, USA) in plasma were measured using commercially available kits.

2.6. Liver Tissue Procedures. Livers were quickly excised from anesthetized rats and were either immediately freeze-clamped in liquid nitrogen, placed in 10% formalin, or placed in ice-cold isolation buffer and then homogenized as previously described [9].

2.7. Fatty Acid Oxidation. Fresh tissue fatty acid oxidation (FAO) using radiolabeled 14C palmitate (Perkin Elmer, Boston, Mass, USA) was performed as previously described [27]. Briefly, both 14CO2, representing complete fatty acid oxidation, and 14C-labeled acid soluble metabolites (14C ASM), representing incomplete fatty acid oxidation, were collected in the previously described trapping device and then counted on a liquid scintillation counter. In addition to the 14C palmitate, the reaction buffer contained a final concentration of 50 μM cold palmitate and 0.5% bovine serum albumin.

2.8. Enzyme Assays. Carnitine palmitoyl transferase-1 (CPT-1) activity, β-hydroxy-acyl-CoA dehydrogenase activity (β-HAD), and citrate synthase activity assays were performed as described previously by our group [27].

2.9. Hepatic Nuclear Isolation. Nuclear extraction of fresh liver tissue was performed using a commercially available kit (Marligen Biosciences, Ijamsville, Md, USA). Briefly, fresh tissue was immediately placed into hypotonic lysis buffer, homogenized, washed, vortexed, and centrifuged. Supernatant containing the cytoplasmic extracts was removed. Each sample was washed by centrifugation until the supernatant was clear. Samples were incubated on ice followed by a 30 min high-speed centrifugation at 4°C. Supernatant was removed representing the nuclear extract and stored at −80°C.

2.10. Hepatic TAG and DAG Analysis. Hepatic fatty acids were extracted using a modified Folch method [28]. Briefly, liver tissue was homogenized in ice-cold Trizma/EDTA buffer and lipids were extracted in chloroform/methanol/acetic acid (2:1:0.15). Extracted lipids were then run on a thin-layer chromatography (TLC) silica plate in a tank containing hexane, diethyl ether, and acetic acid (70:30:1). Triglyceride and diacylglycerol fractions were scraped from the TLC plate and were then methylated by incubating with toluene, methanol, and acetyl chloride (0.5:1.2:0.1) at 100°C for 60 min and separated in hexane. Fatty acid methyl esters were analyzed by gas chromatography (Agilent Technologies, Wilmington, De, USA).

2.11. Liver Histology. Hematoxylin and eosin (H&E) staining was performed on formalin-fixed paraffin-embedded section of liver as described previously to assess lipid vacuolization [29].

2.12. Western Blotting. Western blots were performed to detect protein levels of PPARα (Santa Cruz Biotechnology, Santa Cruz, Calif, USA), PPARδ (Thermo Scientific, Rockford, Il, USA), PPARγ (Cell Signaling, Beverly, CA), SREBP-1c (Santa Cruz Biotechnology), fatty acid synthase (FAS) (Cell Signaling), acetyl coenzyme A carboxylase (ACC) (Cell Signaling), and stearoyl CoA desaturase (SCD)-1 (Alpha Diagnostic International, San Antonio, Tex, USA) as performed previously [9]. Liver samples were homogenized in ice-cold lysis buffer, separated by SDS-PAGE gels, transferred to PVDF membranes, and probed with primary antibodies. Protein bands were quantified using a densitometer and band densities were corrected for total protein loaded by staining with 0.1% amido black (Sigma) as described previously [9].

2.13. Gene Expression. Quantitative RT-PCR was performed as previously described for mRNA expression of PPARα and PPARδ [26]. Briefly, on the day of sacrifice, fresh tissue was collected and placed in RNAlater (Ambion, Austin, Tex, USA) stored at 4°C for 24 h and then stored at −20°C. Liver samples were pulverized in RLT buffer using the QIAGEN TissueLyser system (Valencia, Calif, USA). RNA was then isolated using a commercially available kit (QIAGEN, Valencia, Calif, USA). Reverse transcription and cDNA synthesis were performed according to the manufacturer instructions (Promega, Madison, Wis, USA). TaqMan Master Mix (Applied Biosystems, Foster City, Calif, USA) for gene targets PPARα and PPARδ were loaded into a 96-well microplate along with the cDNA sample (5 μg/μL) and placed into the ABI 7000 Sequence Detection System for polymerization. Results were quantified relative to β-actin. Critical threshold (Ct) values for the housekeeping gene did not differ among groups (data not shown).

2.14. Statistical Analysis. Treatment differences were analyzed by one-way analysis of variance (ANOVA) with main effect significance set at P < 0.05. Significant main effects were then followed up by least significant difference (LSD) post hoc comparisons (SPSS 17.0). Data are presented as means ± SE, and significance was set at P < 0.05.
Values are means ± SE (n = 8). Values with different letter superscripts are significantly different from each other (P < 0.05). Fat pad mass: omental + retroperitoneal fat pads; BW: body weight.

3. Results

3.1. Animal and Serum Characteristics. Final body weight and fat pad mass (omental + retroperitoneal) were significantly different among all groups (Table 1). Body fat percentage was highest in the SED and n3PUFA-SED rats; EX rats had the lowest body fat percentage, over 2.5-fold lower than SED and n3PUFA-SED and 1.6-fold lower than n3PUFA+EX. Fat pad mass mirrored the body fat percentage measures except that n3PUFA-SED was lower than SED. Weekly food intake (grams) was reduced in the EX and n3PUFA+EX groups as compared to SED and n3PUFA-SED. However, when weekly food intake (grams) was normalized to body weight, the EX group consumed more than the SED, n3PUFA-SED, and n3PUFA+EX groups. Weekly caloric intake per body weight was also highest in the EX compared to all other groups. In addition, the SED group consumed more Kcal per body weight than the two groups who received n3PUFA supplementation. All 3 treatment conditions significantly lowered circulating TAG and FFAs compared with SED rats.

3.2. Weekly Run Distance and Body Weight. EX and n3PUFA+EX rats were given access to running wheels at 13 weeks of age. There were no differences in weekly run distances between the two groups (Figure 1(a)). Peak running distances in both groups were approximately 56 and 55 km per week (approximately 8 km per night) at 16 weeks and slowly declined to about 34 and 33 km per week (~5 km per night) by 20 weeks of age. As shown in Figure 1(b), EX and n3PUFA+EX rats showed a decline in body weight the first two weeks following introduction to wheel running and then began gaining weight again while SED and n3PUFA-SED rats continued to gain weight during the 8 weeks of treatment.

**Table 1: Animal and serum characteristics.**

|                      | SED            | EX             | n3PUFA-SED     | n3PUFA+EX      |
|----------------------|----------------|----------------|----------------|----------------|
| Final body weight (g)| 617.1 ± 20.0a  | 462.0 ± 10.5b  | 571.6 ± 10.3c  | 512.1 ± 17.5d  |
| Body fat (%)         | 31.9 ± 0.9a    | 11.3 ± 1.6b    | 29.5 ± 1.1a    | 18.8 ± 1.3c    |
| Fat pad mass (g)     | 33.1 ± 2.0a    | 9.2 ± 1.6b     | 26.4 ± 1.7c    | 16.4 ± 1.8d    |
| Food intake (g)      | 228.2 ± 7.5a   | 192.2 ± 4.0b   | 226.6 ± 5.8a   | 178.5 ± 3.2b   |
| Food wt (g)/BW (g)   | 0.40 ± 0.00a   | 0.45 ± 0.01b   | 0.40 ± 0.00a   | 0.39 ± 0.01a   |
| Caloric intake (kcal)| 947.0 ± 31.0a  | 797.8 ± 16.7b  | 853.2 ± 21.9b  | 672.1 ± 11.9c  |
| Calories (kcal)/BW   | 1.67 ± 0.01a   | 1.84 ± 0.05b   | 1.52 ± 0.02c   | 1.48 ± 0.03c   |
| Triglycerides (mg/dL)| 123.7 ± 17.6a  | 62.1 ± 9.4b    | 59.4 ± 5.4b    | 36.9 ± 4.6b    |
| Free fatty acids (μmol/L)| 422.6 ± 38.4a| 199.3 ± 25.8b  | 263.0 ± 25.3b  | 193.4 ± 26.5b  |
| Glucose (mg/dL)      | 396.0 ± 14.1a  | 255.9 ± 16.0b  | 311.9 ± 45.1b  | 239.2 ± 14.5b  |
| Insulin (ng/mL)      | 15.0 ± 1.9a    | 10.0 ± 1.3b    | 11.8 ± 1.6ab   | 7.6 ± 0.9b     |
| Heart wt/body wt (mg/g)| 2.5 ± 0.10a   | 3.5 ± 0.01b    | 2.7 ± 0.10a    | 3.1 ± 0.10b    |

**Figure 1:** Running distance and body weights. Weekly run distance (a) during the 8 week treatment intervention and weekly body weight (b). Values are means ± SE (n = 8). *EX and n3PUFA+EX are significantly different from SED and n3PUFA-SED (P < 0.05). #EX is significantly different from all groups, and n3PUFA+EX is significantly different than SED and n3PUFA-SED.
3.3. Hepatic Steatosis. Hepatic TAG content from previously studied 13-week-old OLETF-SED rats \( (n = 8) \) was used as a baseline measure of hepatic steatosis allowing us to determine if the 8-week treatments could reverse steatosis. As shown in Figure 2(a), EX rats had lower TAG content at 20 weeks of age as compared to the baseline TAG content of SED-12 wk rats \( (P = 0.03) \), indicating EX effectively treated NAFLD. Although the beneficial effects of EX were partially blunted in the n3PUFA+EX animals, liver TAG content was significantly lower than SED-20 wk \( (P = 0.001) \). Unexpectedly, n3PUFA supplementation in sedentary, hyperphagic rats exacerbated hepatic steatosis above that measured in both SED groups \( (P < 0.05; \ \text{Figure 2}) \). Hepatic DAG content (Figure 2(b)) followed similar trends as TAG content, with EX and n3PUFA+EX both offering protection against the accumulation of DAGs in the liver as compared to SED and n3PUFA-SED. The n3PUFA content eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were measured in the liver and showed that EPA and DHA were incorporated into the liver at a higher level in n3PUFA-fed rats with n3PUFA-SED having the highest content (Figure 2(c)). Representative images of H&E staining
for lipid vacuolization (Figure 2(c)) demonstrate a visual presence of steatosis in n3PUFA-SED and SED rats as shown by the large size and number of lipid droplets, whereas EX and n3PUFA+EX displayed noticeably less lipid droplets.

3.4. Systemic Insulin Sensitivity. Blood glucose and insulin levels were elevated in SED rats as compared to EX, n3PUFA-SED, and n3PUFA+EX (Table 1). Glucose responses to the tolerance test are shown in Figure 3(a). Glucose and insulin AUCs during the IPGTT did not differ between n3PUFA and SED groups, whereas EX rats had significantly lower glucose and insulin AUCs (P < 0.01), and n3PUFA-EX rats only had lower insulin AUCs (P < 0.05) as shown in Figures 3(b) and 3(c).

3.5. Hepatic Mitochondrial Function and Fatty Acid Oxidation. Only the EX group had higher CPT-1 activity than SED (Figure 4(a); P < 0.05). EX and n3PUFA+EX had higher citrate synthase activity followed by SED (Figure 4(e)), and there were no differences among groups in β-HAD activity, though a main effect of treatment groups versus SED approached significance (Figure 4(f); P = 0.059). Rats in the n3PUFA-SED and n3PUFA+EX groups had significantly higher total FAO (14CO2 + 14C ASM) as compared to SED and EX rats (Figure 4(c)). This elevation in total FAO was due to elevated incomplete fatty acid oxidation as shown by higher ASM production (data not shown). Further, in the presence of etomoxir, which inhibits CPT-1 thereby ceasing the transport of long-chain fatty acids to the mitochondria, n3PUFA-SED and n3PUFA+EX had higher rates of FAO than the EX group but not the SED group (Figure 4(d)). This suggests that the dietary intake of n3PUFAs increases extramitochondrial total FAO in both SED and EX conditions. In addition, EX was the only group to have significantly higher complete FAO (14CO2) as compared to SED, n3PUFA-SED, and n3PUFA+EX as shown in Figure 4(b).

3.6. Hepatic Transcription Factors and Fatty Acid Synthesis Pathway. PPARα mRNA expression was significantly lower in EX and n3PUFA+EX (Figure 5(a)). The expression of PPARδ, a transcription factor associated with mitochondrial biogenesis and fatty acid oxidation, was 3-fold higher in EX as compared to SED, n3PUFA-SED, and n3PUFA+EX (Figure 5(b)). However, there were no differences in protein expression of PPARα or δ among groups as shown in Figures 5(c) and 5(d). The nuclear protein content of SREBP-1c did not differ among groups, although there was a trend toward significance (P = 0.09) as seen in Figure 6(a). There also was a trend (P = 0.09) for a main effect for treatment on ACC protein content. However, the EX rats had significantly lower FAS protein content (P = 0.02) as compared to SED, n3PUFA-SED, and n3PUFA+EX. There were no differences in PPARγ protein content among groups (representative western blot shown in Figure 6(f)). EX rats also had the lowest protein content of SCD-1 as compared to the other groups (Figure 6(d)). SCD-1 desaturase index was lowest in EX and highest in n3PUFA-SED rats as estimated by product (16:1) to substrate (16:0) ratio for the enzyme (Figure 6(e)).

4. Discussion

The primary objectives of the current study were to determine if exercise and n3PUFA supplementation, alone or in combination, could effectively treat hepatic steatosis in obese OLETF rats and to determine if these treatment strategies were associated with changes in insulin sensitivity, hepatic mitochondrial FAO, or markers of hepatic lipogenesis. The major findings include the following: (1) exercise treatment reversed preexisting hepatic steatosis in association with elevated hepatic complete FAO and CPT-1 activity, improved systemic insulin sensitivity, and decreased markers of hepatic lipogenesis; (2) n3PUFAs supplemented at 3% of diet increased TAG accumulation in the liver and, when combined with exercise n3PUFAs, did not lead to additive health benefits, but instead partially blunted the beneficial effects of exercise to reduce hepatic TAG accumulation and improve insulin sensitivity. EX was the only treatment that reversed liver TAG content below the pretreatment levels measured in 13-week-old sedentary OLETF animals. It is likely that EX lowered hepatic steatosis in part by slowing gains in body weight and fat mass that normally occur in SED conditions. However, the data also supports the notion that EX induced a hepatic metabolic phenotype that played a role in reducing steatosis as well. Evidence suggests that EX increased both the mitochondrial entry rate (higher CPT-1 activity (Figure 4(a)) and complete catabolism of long-chain fatty acids (increased complete palmitate oxidation to CO2 (Figure 4(b)). The increase in complete oxidation is likely due to a more efficient coupling of increased β-oxidation and TCA cycle flux. EX also lowered extramitochondrial hepatic FAO (oxidation that occurs in the presence of CPT-1 inhibition with etomoxir (Figure 4(d)). Our assumption is that extramitochondrial oxidation is primarily driven by peroxisomes, organelles that break down longer chain fatty acids so that they can then be oxidized in the mitochondria. Peroxisomal activity has previously been shown to be higher in the SED OLETF [30] and when not paired with increased mitochondrial FAO [31–33] could lead to the accumulation of fatty acid metabolites and oxidative stress.

Livers were examined 5 hours after the last exercise bout, opening the possibility that the exercise-induced adaptations were primarily due to the last bout of exercise rather than a result of chronic training. In a previous study from our group, OLETF rats exercised on voluntary running wheels for 2X weeks before we locked the wheels to stop exercise [12]. We then examined hepatic responses 5 hours, 53 hours (2 days), and 173 hours (7 days) after the last exercise bout allowing us to determine if 2 or 7 days of inactivity led to different outcomes than the group studied 5 hours after the last bout. In addition, all 3 groups were also compared to OLETFs who were never provided access to running wheels. Most measures of mitochondrial content were unchanged by 7 days of inactivity and were still higher than sedentary...
livers, while markers of *de novo* lipogenesis (FAS and ACC protein content and malonyl CoA) increased significantly 2 and 7 days after the cessation of exercise. Hepatic TAG did not increase significantly 7 days after the cessation of exercise and remained significantly lower than the content measured in sedentary rats. Thus, these data suggest that some hepatic adaptations to EX treatment are sustained and due to chronic training while others are dependent on the effects of the last bout.

Peripheral insulin resistance is strongly associated with hepatic steatosis. Importantly, high levels of circulating insulin activate SREBP-1c, a master regulator of lipid synthesis. Here we report that EX had the most powerful effects on reducing glucose and insulin levels during the IPGTT. Similar to our previous reports of exercise preventing the development of insulin resistance in the OLETF model [9, 12, 34], here we demonstrate that exercise also can be used as a treatment to improve insulin sensitivity. We also originally hypothesized that EX or dietary n3PUFAs would reduce the mature form of SREBP-1c and would play a key role in downregulating fatty acid synthesis in the liver [16, 18, 35–37]. In addition, we hypothesized that n3PUFAs would activate PPARα and δ, as n3PUFAs are known to be natural ligands and potent activators of PPARs. Surprisingly, the mature, nuclear form of SREBP-1c was not significantly altered by any of the treatments and n3PUFAs did not potently increase expression or protein content of PPARs. However, although there were no changes in SREBP-1c, protein content of FAS and SCD-1, its downstream targets, was dramatically reduced with EX. It is possible that the trend for lowering of nuclear SREBP-1c was enough to lower FAS and SCD-1 or that EX

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**Figure 3:** Insulin sensitivity. Peripheral insulin sensitivity was assessed by intraperitoneal glucose tolerance tests represented as changes in blood glucose over time (a), glucose area under the curve (AUC) (b), and insulin AUC (c). Values are means ± SE (n = 8). *EX is different from other groups,  *n3PUFA+EX is different from SED,  *n3PUFA+EX is different from n3PUFA-SED (P < 0.05). Values with different letter superscripts are significantly different from each other (P < 0.05).
Figure 4: Hepatic fatty acid oxidation. Carnitine palmitoyl transferase (CPT)-1 activity (a), hepatic complete fatty acid oxidation ($^{14}$CO$_2$) (b), hepatic total fatty acid oxidation ($^{13}$CO$_2$ + $^{14}$C ASM) (c), hepatic total extramitochondrial fatty acid oxidation ($^{14}$CO$_2$ + $^{14}$CASM) in the presence of etomoxir (d), citrate synthase activity (e), and β-HAD activity (f). Values with different letter superscripts are significantly different from each other ($P < 0.05$).
lowered these factors through other pathways. Through the conversion of saturated fatty acids to monounsaturated fatty acids, hepatic SCD-1 contributes to abnormal partitioning of fatty acids by reducing FAO and increasing fatty acid synthesis. EX significantly suppressed SCD-1 expression and activity, resulting in lower desaturation of the fatty acid within hepatic TAG which is consistent with our previous results. In contrast, n3PUFA did not impact SCD-1 content and slightly increased activity as shown by the desaturase index. FAS is also a critical step in the conversion of carbohydrates to fatty acids through de novo lipogenesis. All told, EX lowered markers of hepatic lipogenesis, but because this occurred without a clear lowering of nuclear SREBP-1c content, we are unsure these changes are directly linked to reduced circulating insulin and improved systemic insulin sensitivity.

Contrary to our original hypothesis and the results of others, we found that n3PUFA supplementation in SED OLETFs led to increased TAG accumulation in the liver [16, 19, 20, 35, 38]. Although our results are conflicting with a large majority of the literature, it should be noted that the majority of fish oil/n3PUFA research conducted in animals uses excessive amounts (6–60% of energy) that are not likely physiologically relevant [16, 35–41]. Here we used a diet consisting of 3% of total energy from fish oil which is a concentration that can safely be consumed by humans [42]. Consequently, it may require higher dosages of n3PUFAs to yield the health benefits found by others in their rodent models. In addition, we started treatment at 13 weeks of age, an age where OLETFs several obesity-induced comorbidities such as NAFLD, systemic insulin resistance, and increased adiposity are present which may explain the
Figure 6: Lipogenic markers. Protein content of nuclear SREBP-1c (a), ACC (b), FAS (c), SCD-1, and PPARγ (d). Representative blots are shown in (f) for each of the target proteins. Desaturase index is indicative of SCD-1 desaturase activity and is shown by the ratio of 16:1 to 16:0 (e). Values with different letter superscripts are significantly different from each other (P < 0.05).
differences seen between prevention and treatment with n3PUFAs. An alternative and more probable explanation is that dietary consumption of “healthy” fatty acids cannot likely overcome a hyperphagic, chronic state of positive energy balance and daily weight gain in sedentary rats. Excess energy intake, regardless of the source, will likely result in increased adiposity and ectopic fat storage if not matched with increased energy expenditure. Rather, either lowering total energy intake or increasing energy expenditure is likely needed to effectively treat hepatic steatosis. Future experiments are needed to determine if n3PUFA intake would be more effective in reducing steatosis in animals in which the rate of weight gain is reduced by limiting caloric consumption. There are also human clinical implications to these findings. If patients are in a constant positive energy balance and continuing to gain weight, it is unlikely that increasing dietary n3PUFA would lower hepatic fat content.

In conclusion, our study documents the detrimental effects of overeating while remaining sedentary on adiposity, insulin sensitivity, hepatic mitochondrial function, and hepatic steatosis. Moreover, these adverse events occurred regardless of supplementing n3PUFAs in the diet. However, daily exercise effectively treats hepatic steatosis in obese OLETF rats in part by increasing hepatic mitochondrial function and complete fatty acid oxidation, decreasing activation of the lipid synthesis pathway, and improving systemic insulin sensitivity. Furthermore, treatment with n3PUFAs partially blunted the beneficial effects of exercise in this hyperphagic animal model by unknown mechanisms.

**Abbreviations**

β-HAD: Beta-hydroxyacyl-CoA dehydrogenase  
CPT-1: Carnitine palmitoyl-CoA transferase-1  
FFAs: Free fatty acids  
NAFLD: Nonalcoholic fatty liver disease  
OLETF: Otsuka Long-Evans Tokushima Fatty rats  
TAG: Triglycerides  
n3PUFAs: Polyunsaturated fatty acids.

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