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Modalities of exercise training on liver fat accretion and inflammatory markers in ovariectomized rats

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Résumé

Les facteurs de risque des maladies cardiovasculaires, telle, que la détérioration du profil lipidique, deviennent plus prononcés après la ménopause, ce qui fait de la maladie coronarienne, l'une des principales causes de décès chez les femmes ménopausées. Une proportion importante de femmes prennent du poids après la ménopause en particulier dans la région abdominale entraînant par conséquent des perturbations métaboliques. Des données récentes suggèrent également que l'absence des œstrogènes observée à la ménopause favorise le développement de la stéatose hépatique. Cette dernière a été incriminée pour incriminée dans le développement de la résistance à l'insuline, et est de ce fait considérée comme une composante hépatique du syndrome métabolique. Il est impératif d'établir des stratégies visant à contrecarrer l'accumulation de graisse dans le foie et l'accroissement du tissu adipeux chez les femmes ménopausées, en tenant compte que l'utilisation de l'hormonothérapie substitutive est de nos jours moins soutenue. Les quatre études de la présente thèse ont été conduites pour tenter de fournir des informations sur le traitement et la prévention de l'augmentation de la masse graisseuse et de la stéatose hépatique qu'entraîne la suppression des œstrogènes, à travers les modifications du mode de vie (diète et exercice physique) chez la rate ovariectomizée (Ovx); un modèle animal de la ménopause.

Dans les deux premières études nous nous sommes concentrés sur l'augmentation de la masse graisseuse et sa reprise suite à une perte de poids. Dans la première étude, nous avons montré que les rates Ovx qui ont suivi un programme de restriction alimentaire (FR) ont diminué significativement ($P < 0.01$) leur poids corporel, leur contenu en graisses intra-abdominales ainsi que leurs triacylglycérols (TAG) hépatiques, comparativement aux rates Ovx nourries à la diète normale. De plus, l'entraînement en résistance (RT) a prévenu la reprise de poids corporel ainsi que l'accroissement du tissu adipeux et l'accumulation de lipides dans le foie des rates Ovx, après l'arrêt du régime amaigrissant. Les résultats de la deuxième étude ont confirmé l'efficacité de la restriction alimentaire associée à

l'entraînement en résistance (FR + RT) dans la réduction du poids corporel, des lipides dans le foie et le tissu adipeux chez les rates Ovx. Tenant compte des résultats de notre première étude, l'entraînement en résistance seulement a constitué un atout pour atténuer le poids corporel et la masse grasse reprise par les rates Ovx suite à un programme de perte de poids (FR + RT); bien que l'impact ait été moindre comparé au maintien seul de la restriction alimentaire. De la même manière que la supplémentation en œstrogènes, les résultats de la troisième étude indiquent que l'entraînement en endurance mené concurremment avec l'ovariectomie a significativement atténué l'accumulation de lipides dans le foie ainsi que dans le tissu adipeux. Toutefois, l'entraînement en endurance effectué avant l'ovariectomie n'a pas protégé contre l'accumulation des graisses qu'entraîne l'ovariectomie, si celui-ci est interrompu après l'ovariectomie. Enfin, pour compléter les résultats antérieurs, nous avons montré dans la quatrième étude que l'expression des gènes impliqués dans la synthèse de lipide; SREBP-1c, SCD-1, ChREBP, et ACC dans le foie a augmenté après le retrait des œstrogènes, tandis qu'une diminution ($P < 0.01$) des niveaux d'ARNm de PPAR-α a été observée. De plus, l'expression hépatique des gènes des cytokines pro-inflammatoires incluant IKKβ, IL-6 ainsi que le contenu protéinique de NF-κB étaient augmentés ($P < 0.01$) chez les rates Ovx par rapport aux rates ayant subi une Ovx simulée (Sham). Toutes ces perturbations ont été améliorées avec la supplémentation en œstrogènes seulement, ainsi qu'avec l'entraînement en endurance seulement.

Dans l'ensemble, nos résultats indiquent que l'exercice physique (en résistance ou en endurance) a un impact significatif sur la réduction de l'accumulation des lipides dans le foie et dans le tissu adipeux des rates Ovx. De plus, chez les rates Ovx, l'entraînement en endurance mimerait les effets des œstrogènes sur l'expression des gènes impliqués dans l'accumulation de lipides et l'inflammation préclinique dans le foie.

Mots-clés : Œstrogènes, Obésité ménopausique, Reprise de poids et de graisse, Foie gras, Restriction alimentaire, Entraînement en résistance, Entraînement en endurance, Bio-marqueurs de l'inflammation, Ovariectomie (Ovx), Rat

Abstract

Cardiovascular disease risk factors, such as lipid profile deterioration, become more pronounced after menopause making coronary heart disease a leading cause of death among postmenopausal women. A large proportion of women after menopause gain weight especially in the abdominal region resulting in several metabolic disturbances. Recent evidence also suggests that loss of estrogen function in menopause is associated with the development of a state of hepatic steatosis. Excessive fat accumulation in hepatocytes has been shown to play an important role in the development of insulin resistance and is even considered as a hepatic component of the metabolic syndrome. There is an important need to establish strategies to counteract fat accumulation in adipocyte and liver in postmenopausal women specifically considering the fact that utilization of hormone replacement therapy is now less supported. The four studies of the present thesis have been conducted in an attempt to provide information on the treatment and prevention of estrogen withdrawal-induced fat mass and hepatic steatosis via lifestyle modifications (diet and exercise training) in an ovariectomized (Ovx) rat model of menopause.

In the first two studies we focused on fat mass gain and regain following weight loss. In study 1, we showed that food restriction program (FR) decreased ($P < 0.01$) body mass, intra-abdominal fat pad weight, and liver triacylglycerol (TAG) levels as compared to normally fed Ovx rats. Moreover, resistance training program (RT) was useful in preventing body weight as well as adipose tissue and liver fat regain in Ovx rats, following diet-induced weight loss. Results of study 2 confirmed the efficiency of the FR + RT program in reducing body weight as well as liver and adipocytes fat accretion in Ovx rats. In line with the findings of our first study, continuation of only RT constituted an asset to attenuate body weight and fat mass regain in Ovx rats following a FR + RT weight loss program, although the impact was less than maintaining FR alone. Similar to estrogen supplementation, results of study 3 indicated that endurance exercise training conducted concurrently with the induction of ovariectomy significantly attenuated liver and adipocyte

fat accumulation. However, an endurance exercise training state acquired before ovariectomy did not provide any protective effects against ovariectomy-induced fat accumulation if exercise is discontinued after the ovariectomy. Finally, complementing previous findings we showed in study 4 that liver gene expressions of transcription factors SREBP-1c and ChREBP along with downstream lipogenic enzymes SCD-1 and ACC were increased with estrogens withdrawal conversely to reduced PPAR-α mRNA levels ($P < 0.01$). Furthermore, gene expressions of pro-inflammatory cytokines including IKKβ and IL-6 as well as protein content of NF-κB were higher ($P < 0.01$) in the liver of Ovx than in Sham animals. All of these responses were corrected with estrogen supplementation alone as well as with endurance exercise training alone in Ovx rats.

On the whole, our results indicate that exercise training (resistance or endurance) has a significant impact on reducing fat accumulation in liver and adipocytes in Ovx rats. In addition, it seems that endurance exercise training in Ovx rats stimulates estrogenic-like effects on the expression of genes involved in lipid accumulation and sub-clinical inflammation in the liver.

Keywords: Estrogen, Menopausal obesity, Weight and fat regain, Hepatic steatosis, Food restriction, Resistance training, Endurance exercise training, Inflammatory bio-markers, Ovariectomy (Ovx), Rat

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Abbreviations

ACC : Acetyl-CoA carboxylase

AMPK : AMP-activated protein kinase

CVD : Cardiovascular disease

CHD : Coronary artery disease

CRP : C-reactive protein

ChREBP : Carbohydrate response element-binding protein

E2 : 17β-estradiol (estrogen)

ER : Estrogen receptor

FFA : Free fatty acid

FAS : Fatty acid synthase

FR : Food restriction

HDL : High density lipoprotein

HRT : Hormone replacement therapy

HSL : Hormone sensitive lipase

IKKβ : Inhibitor of kappa B kinase beta

IL : Interleukin

IRS : Insulin receptor substrate

LDL : Low density lipoprotein

LPL : Lipoprotein lipase

MCP-1 : Monocyte chemotactic protein 1

NAFLD : Nonalcoholic fatty liver disease

NF-κB : Nuclear factor-kappa B

Ovx : Ovariectomy

PAI-1 : Plasminogen activator inhibitor 1

PGC : Peroxisome proliferator-activated receptor-γ coactivator

PPAR : Peroxisome proliferator-activated receptor

PKC : Protein kinase C

RT : Resistance training

SCD-1 : Stearoyl coenzyme A desaturase 1

SREBP-1c : Sterol regulatory element-binding protein-1c

T2DM : Type 2 diabetes mellitus

TAG/TG : Triacylglycerole

TNF : Tumor necrosis factor

VLDL : Very low density lipoprotein

To all my families (old, new, future)
especially to my mother

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Introduction

One of the subpopulations in which the prevalence of obesity and overweight is growing rapidly is postmenopausal women. Although it is not yet clear whether the menopausal transition itself leads to weight gain, it is known that the physiological withdrawal of estrogen brings about changes in fat distribution that increase the risk for the metabolic syndrome, diabetes, and cardiovascular disease [Dubnov-Raz, Pines et al. 2007]. In fact, with arrival of menopause, women experience an increase in body weight and alterations in body composition, with a tendency for intra-abdominal (visceral or central) fat accumulation [Brochu, Starling et al. 2000]. Increased intra-abdominal fat is strongly associated with insulin resistance and cardiovascular complications, while the same amount of lower body fat seems to have a protective effect [Kopelman 2000; Okura, Nakata et al. 2004]. Remarkably, postmenopausal women tend to accumulate fat outside the adipose tissue (mainly in the liver) referred to as ectopic lipid deposition, which may be the cause of deleterious metabolic complications [Volzke, Schwarz et al. 2007; Kotronen and Yki-Jarvinen 2008]. On the other hand, there is growing evidence of the metabolic and cardiovascular impact of liver lipid infiltration [Johnson, Sachinwalla et al. 2009] and interventions which reduce hepatic fat concentration are often accompanied with significant improvements in metabolic function such as insulin resistance and cardiovascular metabolic disturbances [Petersen, Dufour et al. 2005]. These observations highlight the importance of understanding the molecular and physiological mechanisms that underlie menopause-associated obesity and metabolic dysregulation. Therefore, it is relevant to investigate possible strategies and their underlying mechanisms for the prevention/treatment of adipocyte and liver fat accumulation in the estrogen-deficient state.

The four studies presented in this thesis have been conducted in an attempt to provide information on the treatment and prevention of estrogen withdrawal-induced fat mass increase and hepatic steatosis by lifestyle modifications (diet and exercise training) in ovariectomized (Ovx) rat model of menopause. Rodent ovariectomy is one approach to modeling human menopause and studying the metabolic consequences of loss of ovarian function. Studies in rodents consistently demonstrate that Ovx promotes obesity and its

metabolic complications. Using the Ovx model, we addressed several questions regarding regulation of adipocytes and liver fat accumulation.

In the first study, we tested the hypothesis that substituting food restriction (FR) by resistance training (RT) after a period of weight loss would maintain the decrease in fat accumulation in liver and adipose tissue that occurs with weight loss in Ovx rats. In line with this approach, the second study investigated the effect of maintaining RT or FR on body weight regain, fat mass, and liver lipid infiltration in estrogen deficient animals previously submitted to a FR + RT weight loss program. An interesting question related to exercise and estrogen withdrawal is whether women who exercise regularly during their reproductive period are protected against the deleterious metabolic effects of menopause. Therefore, in the third study, we addressed this question using an animal model that allowed us to test a complete design of trained and untrained animals before and after withdrawal of estrogens. In continuation with our third study, the aim of the fourth study was to test the hypothesis that exercise training reduces the expression of key molecules involved in lipid synthesis while favoring the expression of molecules involved in fat oxidation. The second objective of this last study was to investigate the effects of ovariectomy and exercise training on gene expression of inflammatory markers in the liver.

This thesis comprised seven chapters. The first section of chapter 1 presents a review of literature on the emergence of metabolic syndrome (intra-abdominal fat) and hepatic steatosis in the postmenopausal hormonal state, and their treatment and prevention by lifestyle modifications (exercise training). In the second part, a review on the pathogenic role of sub-acute inflammation in obesity and insulin resistance is presented. Chapters 2-5 introduce the experimental studies of this thesis that are presented according to the format required by the journals in which they are published or have been submitted to and have the references provided at the end of each study. Finally, chapter 6 presents a general discussion and conclusion on the studies presented in this dissertation. Chapter 7 presents thesis references.

Chapter 1: Review of literature

Emergence of metabolic syndrome and hepatic steatosis in menopausal hormonal state: treatment and prevention by exercise training

Cardiovascular disease in postmenopausal women

Gender differences in the development of cardiovascular disease (CVD) are well documented. Female gender is comparatively protected against CVD in the reproductive age range [Loria, Lonardo et al. 2008]. However, coronary heart disease (CHD) is a main and leading cause of death in women [Wingo, Calle et al. 2000]. Early epidemiological studies indicated higher incidence of the disease in postmenopausal women compared to women of reproductive age [Gordon, Kannel et al. 1978; Rosenberg, Hennekens et al. 1981; Colditz, Willett et al. 1987; Matthews, Meilahn et al. 1989]. Accordingly, cardiovascular diseases are more prevalent in men than in premenopausal women, but the incidence increases sharply in postmenopausal women [Wenger, Speroff et al. 1993]. Menopause is characterized by the progressive reduction of estrogens resulting to cessation of menses [Mastorakos, Valsamakis et al. 2010]. Strategies to prevent CVD in this population should therefore be a principal objective for healthcare providers.

Based on above evidence, the hypothesis that estrogens have protective effect against atherosclerosis has been put forward. Studies that have investigated the role of age at menarche and the calculated total lifetime exposure to endogenous estrogen, indicate that endogenous estrogens appear to play a protective role for the cardiovascular system [de Kleijn, van der Schouw et al. 2002; Jansen, Temme et al. 2002; Saltiki, Doukas et al. 2006]. These studies conclude that shorter lifetime exposure to endogenous estrogens is an

important risk factor for the presence and the severity of coronary heart disease. It is known that estrogens exert several protective effects on the cardiovascular system such as favorably modifying the lipid profile by increasing high density lipoprotein (HDL) and lowering low density lipoprotein (LDL) while also improving endothelial function [Lieberman, Gerhard et al. 1994]. The lack of these estrogenic effects at the time of menopause results in deleterious metabolic changes that not only negatively affect lipids but also fat redistribution and insulin resistance [Seed 2002]. Therefore, although we have to be careful on the effect of age, menopause can be considered a risk factor for CVD because estrogen withdrawal has a negative effect on cardiovascular functions and metabolism. Menopause negatively impacts upon many traditional risk factors for CVD, including changes in body fat distribution from a gynoid to an android pattern, reduced glucose tolerance, abnormal plasma lipids, increased blood pressure, endothelial dysfunction and vascular inflammation [Rosano, Vitale et al. 2007]. Based on these data, hormone replacement therapy in postmenopausal women was the first line prescription by physicians for many years and numerous observational studies suggested a cardiovascular benefit in women taking postmenopausal hormone replacement therapy [Psaty, Heckbert et al. 1994; Sidney, Petitti et al. 1997; Grodstein, Manson et al. 2000]. However, in recent years, large prospective and randomized trial studies such as the Women's Health Initiative (WHI) reported no CHD benefit by hormone replacement therapy and even suggested a possible increased incidence of CVD [Rossouw, Anderson et al. 2002]. Although no specific mechanism has yet fully explained this paradox, several potential adverse consequences from estrogen therapy relative to CHD risk have been proposed, such as elevations in TAGs and C-reactive protein (CRP) along with increased likelihood of thrombus formation; all of which have been implicated in increasing CHD risk in women [Welty 2001; Alexander and Clearfield 2006]. Moreover, from a clinical standpoint, the protection conferred by initiating hormone replacement therapy soon after the menopause is small [2006]. Therefore, it seems that prevention of CVD or CHD in postmenopausal women have to mostly rely on well-established interventions such as diet and exercise

which should be vigorously emphasized by health care providers at the time of menopause [2006; Alexander and Clearfield 2006].

Menopausal obesity and the emergence of metabolic syndrome

The prevalence of obesity and type 2 diabetes mellitus (T2DM) is rapidly increasing worldwide. The public health consequences of this situation are devastating and recognized by practically every major international health organization. Obesity is first and foremost a problem of energy imbalance (energy intake exceeds energy expenditure); and unfortunately, women who can expect to live almost more than a third of their lives after menopause; are disproportionately affected by obesity and its co-morbidities such as aforementioned CVD. Review of the relevant literature and results from recent clinical trial studies indicate that 60% of postmenopausal women are considered overweight and obese and 43% present the metabolic syndrome [Ford, Giles et al. 2002]. In addition, postmenopausal status is associated with a 60% increased risk of the metabolic syndrome, even after adjusting for confounding variables, such as age, body mass index, household income, and physical inactivity [Park, Zhu et al. 2003]. Menopausal obesity-related CVD become a leading cause of morbidity and mortality in women after fifty years of age [Simoncig-Netjasov, Vujovic et al. 2008]. In parallel there is an increased prevalence of cardiometabolic abnormalities in the transition from pre- to postmenopause such as increased central (intra-abdominal/visceral/abdominal) body fat, a shift toward a more atherogenic lipid profile, increased blood pressure, and glucose intolerance along with reduced insulin sensitivity and high prevalence of nonalcoholic fatty liver disease (NAFLD); elucidating the noticeable increase of rate in CVD after menopause [Carr 2003; Clark 2006]. It has been suggested that there is a metabolic syndrome resulting from the menopause due to estrogen deficiency, as many of the risk factors are more prevalent in postmenopausal women [Kaaja 2008]. Eshtiaghi *et al.* recently reported that menopause can be a predictor of metabolic syndrome independent of age [Eshtiaghi, Esteghamati et al.

2010]. The features of the metabolic syndrome include the accretion of visceral adiposity, insulin resistance, hypertension, and dyslipidemia (hypertriglyceridemia, reduced HDL, and increased small dense LDL particles) (Table 1) [Despres 1993]. The emergence of these risk factors may be a direct consequence of ovarian failure or alternatively, an indirect result of the metabolic cost of central fat redistribution with estrogen deficiency [Carr 2003]. Nevertheless, the exact mechanism linking menopausal hormonal context and its resulting visceral adiposity to the downstream metabolic diseases remains unclear.

Menopause, weight gain and fat redistribution

The rate of weight gain during the menopausal period is not consistent between studies [Panotopoulos, Raison et al. 1997]. While it is still unclear whether the menopause transition itself brings about weight gain [Crawford, Casey et al. 2000; Dubnov-Raz, Pines et al. 2007], there is good evidence that menopause is associated with weight gain and changes in fat distribution that increase the risk of cardiovascular diseases [Astrup 1999; Milewicz, Demissie et al. 2003; Genazzani and Gambacciani 2006]. Rosano *et al.* reported that postmenopausal women tend to gain weight from the first year of menopause and experience a redistribution of body fat from a gynoid to an android pattern [Rosano, Vitale et al. 2007]. There are two patterns of fat distribution: accumulation of fat centrally as intra-abdominal fat (named android or apple shape); and accumulation of fat peripherally in the gluteo-femoral region (named gynoid or pear shape). Apple shape/android/intra-abdominal/central/visceral fat deposition is associated with a higher risk of hypertriglyceridemia, insulin resistance, diabetes, and CVD, independently of overall obesity [Kannel, Cupples et al. 1991; Despres 1993]. It seems that estrogen promotes the accumulation of gluteo-femoral fat [Krotkiewski, Bjorntorp et al. 1983]. This may, at least partially, explains that fluctuations in reproductive hormone concentrations throughout women's lives uniquely predispose them to excess weight gain. For example, menopause is

Table 1. Taken from [Despres 1993] Features of the metabolic syndrome.

1. Central obesity 2. Insulin resistance 3. Dyslipidemia a. Elevated TG b. Small dense LDL particles c. Reduced HDL 4. High blood pressure 5. Hypercoaguable state 6. Pro-inflammatory state

one of the critical periods of a woman's life during which weight gain and onset or worsening of obesity is favored [Pavon de Paz, Alameda Hernando et al. 2006]. Longitudinal and review of cross-sectional studies support the notion that the menopause transition, independently of aging process and total body fatness, is associated with an increase in abdominal and visceral adipose tissue accumulation [Tchernof, Calles-Escandon et al. 1998]. Alterations in regional adipose tissue metabolism along with positive energy imbalance resulting from hormonal changes of the menopause transition may be potential mechanisms for the menopause related acceleration in abdominal fat accumulation [Guthrie, Dennerstein et al. 2003] (Figure 1). Moreover, Lovejoy *et al.* in their recent observational-longitudinal study with annual measurements for 4 years reported that menopause onset is associated with reduced energy expenditure (both basal and physical activity) and fat oxidation that can predispose to obesity (total and visceral abdominal fat) if lifestyle changes are not made [Lovejoy, Champagne et al. 2008]. A menopause related decline in fat-free mass (muscle) is also reported which may be responsible for a decrease in energy expenditure [Colombel and Charbonnel 1997; Panotopoulos, Raison et al. 1997]. Intra-abdominal adipose tissue is thought to be the most important determinant for the constellation of metabolic disturbances, termed metabolic syndrome. Women with high amounts of visceral fat have an excess of cardiovascular mortality and associated metabolic abnormalities [Lapidus, Bengtsson et al. 1984]. Therefore, it is not surprising that review of the relevant literature and results from recent clinical trials indicate that metabolic syndrome may occur in at least 40% of postmenopausal women, which is largely determined by overweight status and obesity [Lobo 2008]. The prime emphasis in management of the metabolic syndrome and the prevention of CVD is to reduce underlying modifiable risk factors through lifestyle changes [Kaaja 2008]. Consequently, almost all concerned review studies support the importance of focusing on postmenopausal women, with the goal of weight reduction, increasing physical activity and encouraging healthy dietary choices to prevent weight and visceral fat gain in menopause transition [Sowers, Zheng et al. 2007; Kaaja 2008; Lobo 2008; Lovejoy, Champagne et al. 2008].

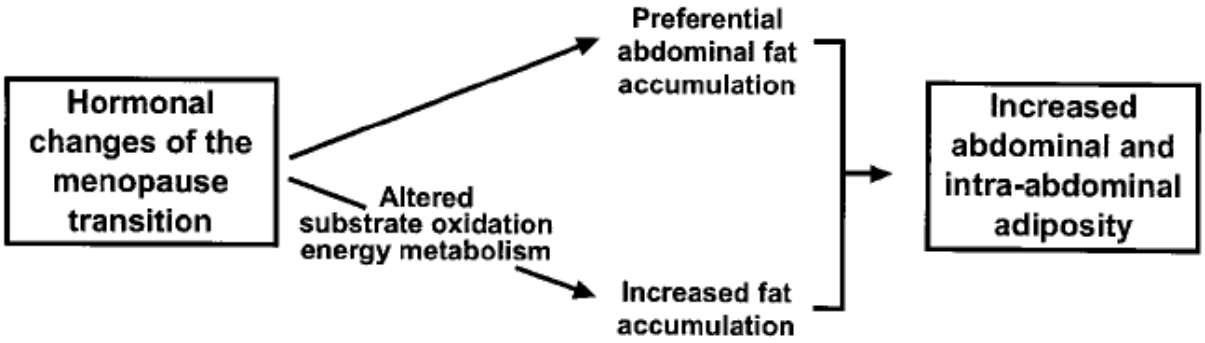


Figure 1. Potential mechanisms explaining menopause-related increases in abdominal and intra-abdominal adiposity.

Menopause and hepatic steatosis

Non-alcoholic fatty liver disease (NAFLD)

Liver lies below the diaphragm in the thoracic region of the abdomen. This organ plays a major role in metabolism and has a wide range of functions including production of biochemicals necessary for digestion, glycogen storage, decomposition of red blood cells, protein synthesis, hormone production, and detoxification [Maton, Hopkins et al. 1993]. Liver is particularly vulnerable to ectopic fat accumulation [Bruce and Byrne 2009] that could result in NAFLD characterized by hepatic lipid accumulation in the absence of significant alcohol consumption. NAFLD is the most frequent chronic liver disease in Western countries [Angulo 2002] and its incidence in both adults and children is rapidly rising in conjunction with the burgeoning epidemics of obesity and T2DM [Stein, Dong et al. 2009]. NAFLD is defined as fatty infiltration of the liver exceeding 5 to 10% by weight [Salt 2004]. It includes a wide spectrum of disorders ranging from simple steatosis described by hepatic lipid accumulation in the form of triglyceride (TG) to nonalcoholic steatohepatitis (NASH) described by the association of lipid accumulation with evidence of hepatocyte injury, inflammation and different degrees of fibrosis [Brunt and Tiniakos 2005]. NASH can also progress to cirrhosis and hepatocellular carcinoma. NAFLD is now considered the hepatic manifestation of metabolic syndrome and has insulin resistance as its feature [Musso, Gambino et al. 2003; Musso, Gambino et al. 2008]. Moreover, new evidence suggests that NAFLD is becoming a risk factor for diabetes and CVD; independently of insulin resistance, metabolic syndrome, plasma lipid levels, and other usual risk factors [Chitturi and Farrell 2007; Alkhouri, Tamimi et al. 2009]. It was reported that hepatic steatosis by itself is associated with a pro-atherogenic lipid profile [Cali, Zern et al. 2007] and increased production of pro-inflammatory markers [Wieckowska, Papouchado et al. 2008]. In support of this, recent epidemiological studies suggest that NAFLD may be dynamically involved in the pathogenesis of CVD, potentially through the increased release of pro-atherogenic markers from liver mainly inflammatory cytokines

[Targher, Marra et al. 2008]. Loria and *et al.* showed that many well-defined metabolic, haemodynamic, hormonal, pro-thrombotic and pro-inflammatory CVD risk factors play a major role in the complex pathophysiology of NAFLD (Table 2) [Loria, Lonardo et al. 2008]. Moreover, they conclude that lipotoxicity derived from NAFLD represents a potential biological mechanism accounting for increased CVD risk, and there appears to be a close link between deranged energy homeostasis, inflammatory changes in adipose and liver tissues and molecular mediators of atherogenesis.

On the other hand, NAFLD is involved in whole-body insulin resistance and dyslipidemia, although whether insulin resistance is a consequence of liver ectopic fat deposition or *vice versa* remains an unanswered question [Bruce and Byrne 2009]. Some researchers have proposed that with insulin resistance, the combination of increased plasma glucose and free fatty acids concentrations promote hepatic fatty acid synthesis and impair β-oxidation leading to hepatic steatosis [Marchesini, Brizi et al. 1999; Sanyal, Campbell-Sargent et al. 2001]. On the contrary, other investigators have projected that liver fat accumulation and hepatic insulin resistance can happen without the development of peripheral insulin resistance [Kraegen, Clark et al. 1991; Kim, Fillmore et al. 2001]. However, when considerable hepatic steatosis occurs, liver becomes insulin resistant and overproduces both glucose and very low density lipoprotein (VLDL) leading to hyperglycemia, hypertriglyceridaemia, and decreased HDL concentrations [Kotronen and Yki-Jarvinen 2008]. Moreover, the results of Samuel *et al.* support the hypothesis that hepatic steatosis leads to hepatic insulin resistance by stimulating gluconeogenesis and activating protein kinase C-ε (PKC-ε) and c-Jun N-terminal protein kinase 1 (JNK1), which may interfere with tyrosine phosphorylation of insulin receptor substrate 1 and 2 (IRS-1 and IRS-2) and impair the ability of insulin to activate glycogen synthase [Samuel, Liu et al. 2004].

The exact pathogenesis of hepatic lipid accumulation seems to be very complex and only partially understood. Nevertheless, it is a condition usually associated with obesity (particularly central abdominal obesity), diabetes, and insulin resistance. On the whole, the

Table 2. Taken from [Loria, Lonardo et al. 2008] Possible pathophysiological bases for an association between NAFLD and accelerated atherosclerosis.

Factor	Atherosclerosis	NAFLD
With a genetic predisposition		
Atherogenic hyperlipidaemia	Associated with high LDL, VLDL and low HDL	Prevalence of NAFLD most elevated in mixed hyperlipidaemia with increased ALT
Arterial hypertension	Associated and partially reversible with a decrease in hypertension	Patients with hypertension have a higher prevalence of NAFLD
Hyperhomocysteinaemia	Associated	Evidence from animal and human studies, including HCV steatosis
T2DM	Strongly associated	Very common in T2DM and a risk factor for the development and progression of NAFLD
Abdominal obesity	Strongly associated	Associated with abdominal obesity and a predictor of liver fibrosis
Prothrombotic state	Association with fibrinogen, PAI-1, Factor VII, Factor VIII, platelet reactivity and others	Associated with fibrinogen, PAI-1, Factor VII, Factor VIII and decreased tissue-type plasminogen activator
Systemic inflammation	Associated with CRP and other acute-phase proteins	Major determinant for the development of NAFLD
Metabolic syndrome and insulin resistance	Strongly associated	Very common in MS and a risk factor for the development and progression of NAFLD
Male gender	Men <60 years of age are twice as likely to be affected compared with women	Female gender protected in the reproductive age range
Environmental		
High-fat diet	Strong association with lifestyle	Reported in NAFLD and impaired postprandial lipid metabolism
Cigarette smoking	Strongly associated and reversible by stopping	Preliminary evidence
Low antioxidants	Findings not conclusive	Findings not conclusive
Sedentariness	Independent association	Associated with NAFLD; exercise is recommended as a treatment

The left-hand column lists the widely accepted genetic and environmental risk factors for atherosclerosis. Interestingly, evidence is mounting that the same factors also play a role in the development of NAFLD. LDL, low density lipoprotein; VLDL, very-LDL; HDL, high density lipoprotein; ALT, alanine aminotransferase; HCV, hepatitis C virus; T2DM, type 2 diabetes mellitus; PAI-1, plasminogen activator inhibitor-1; CRP, C-reactive protein.

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) [Musso, Gambino et al. 2009]. Excessive fat accumulation in the liver can occur as a result of: (i) increased fat delivery into the liver (dietary fatty acids and plasma non-esterified fatty acids derived from adipose tissue), (ii) increased fat synthesis in liver, (iii) reduced fat oxidation, and (iv) reduced fat export in the form of VLDL (see Figure 2 for an overview). 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 [Petta, Muratore et al. 2009]. Therefore many genes, related not only to fat accumulation but also to different mechanisms implicated in the disease progression, have been evaluated, and some polymorphisms capable of increasing the severity of the disease have been identified (Table 3) [Wilfred de Alwis and Day 2007].

According to Petta *et al.* [Petta, Muratore et al. 2009] body fat, insulin resistance, oxidative stress and mitochondrial dysfunction, cytokine/adipokine interplay, and apoptosis are risk factors of NAFLD. Most interestingly, recent data indicates that intra-abdominal (visceral) fat likely plays a pivotal role in the pathogenesis of NAFLD [Thomas, Hamilton et al. 2005] affecting all above mentioned risk factors. In effect, visceral fat acts as an endocrine storage organ, secreting different molecular mediators such as FFA, adiponectin, leptin, TNF, IL-6, etc; and participating directly in NAFLD pathogenesis in different ways, dependently or independently of insulin resistance, therefore contributing to liver fat accumulation [Ronti, Lupattelli et al. 2006]. To date, the most effective treatments of NAFLD are lifestyle changes (diet, weight reduction, and exercise) [Williams, Sander et al. 2006]. However, the front-line therapy with lifestyle modifications resulting in weight loss through decreased caloric intake and exercise is often difficult to maintain on a long term basis [Stein, Dong et al. 2009]. Therefore, information regarding fat accumulation in liver and adipocytes is needed to establish the most effective strategies to prevent and treat NAFLD and to counteract the deleterious metabolic effects of NAFLD.

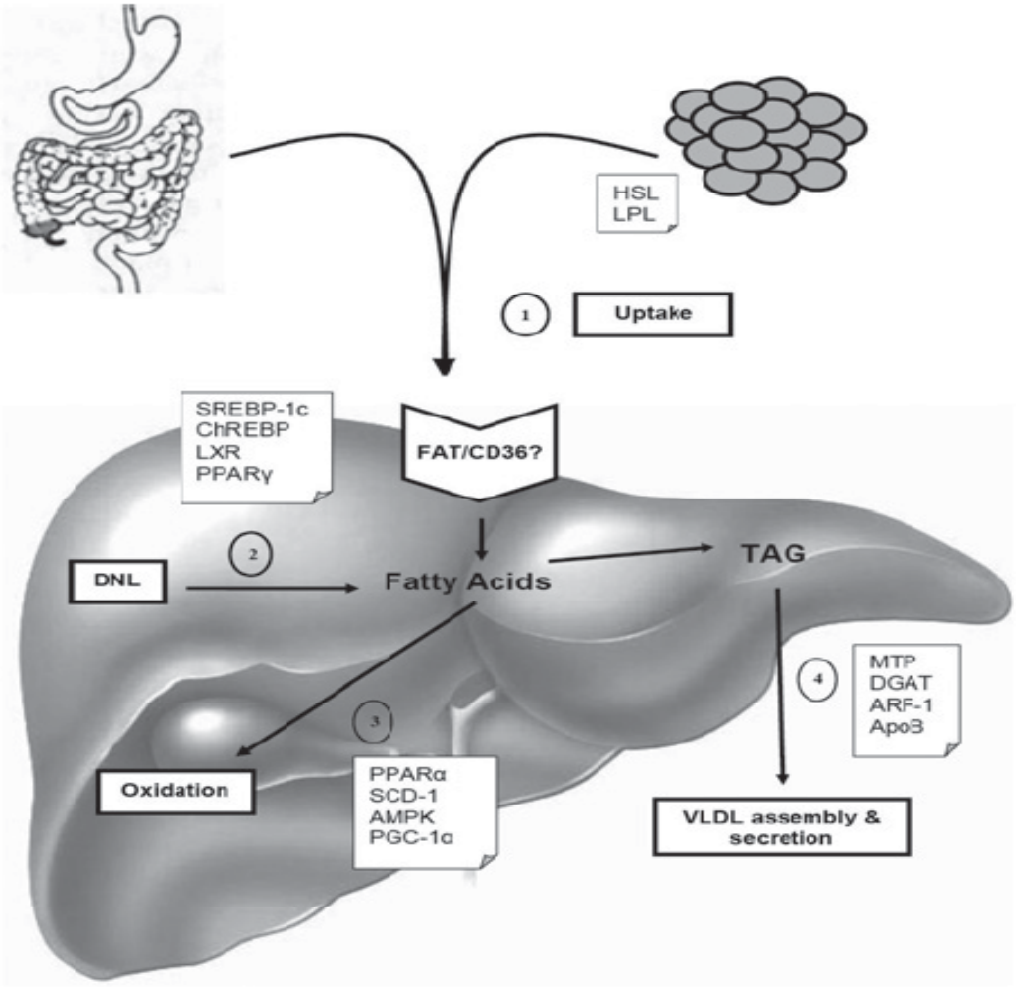


Figure 2. Taken from [Lavoie and Gauthier 2006] Overview of the four main pathways involved in the development of nonalcoholic hepatic steatosis, and their regulatory factors. Nonalcoholic hepatic steatosis is characterized by (1) an increase in the uptake of lipids by the liver, (2) an increase in hepatic de novo lipogenesis (DNL), and an insufficient elimination of excess liver triacylglycerols (TAGs) by means of (3) hepatic lipid oxidation and (4) very low density lipoprotein (VLDL) assembly and secretion. HSL, hormone-sensitive lipase; LPL, lipoprotein lipase; FAT/CD36, fatty acid translocase/cluster of differentiation 36; SREBP-1c, sterol-regulatory-element-binding protein 1c; ChREBP, carbohydrate-response-element-binding protein; LXR, liver X receptors; PPAR,

peroxisomal proliferator-activated receptors; SCD-1, stearoyl-CoA desaturase-1; AMPK, AMP-activated protein kinase; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator-1 alpha; MTP, microsomal transfer protein; DGAT, diacyglycerol acyltransferase; ARF-1, ADP-ribosylation factor 1; ApoB, apolipoprotein B.

Table 3. Taken from [Wilfred de Alwis and Day 2007] Genes potentially involved in the pathogenesis of nonalcoholic fatty liver disease (NAFLD).

Group of genes	Examples
Genes involved in liver fat synthesis	RXR LXR SREBP ChREBP, Fatty acid synthase (FAS) Acetyl CoA carboxylase (ACC) Leptin, Adiponectin
Genes involved in liver fat export	Apolipoprotein B MTP
Genes involved in fatty acid oxidation	Adiponectin PPAR-α Acil CoA oxidase Carnitine palmitoyl transferase (CPT)
Genes involved in insulin resistance	Adiponectin Insulin receptor genes PPAR-γ RBP4 TNF-α IL-6 SOCS PPA2
Genes involved in oxidative stress	Adiponectin, TNF-α SOD GST GSH TLR MnSOD MAO
Genes for adipokines and their receptors	Adiponectin, Leptin, Resistin RBP4, AFABP Adiponectin receptors, Leptin receptors
Genes for cytokines and their receptors	TNF-α IL-6 IL-10 MCP1 TNFR
Genes involved in fibrogenesis	Adiponectin, Leptin Angiotensinogen Steroids KLF6, TGF-β, CTGF MMP, TIMP

RXR, retinoid X receptor; LXR, liver X receptor; SREBP, sterol responsive element binding protein; ChREBP, carbohydrate responsive element binding protein; MTP, microsomal triglyceride transfer protein; PPARα, peroxisome proliferator-activated receptor α; PPARγ, peroxisome proliferator-activated receptor γ; RBP4, retinol binding protein 4; TNFα, tumor necrosis factor α; IL-6, interleukin-6; SOCS, suppressor of cytokine signaling; PPA2, protein phosphatase A2; SOD, superoxide dismutase; GST, glutathione transferase; GSH, glutathione peroxidase; TLR, Toll-like receptor; MnSOD, manganese superoxide dismutase; MAO, monoamine oxidase; AFABP, adipocyte fatty acid binding protein; IL-10, interleukin-10; MCP1, monocyte chemoattractant protein-1; TNFR, tumor necrosis factor receptor; KLF6, Kupper-like factor 6; CTGF, connective tissue growth factor; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of matrix metalloproteinase.

NAFLD in postmenopausal women

Gender may influence the incidence and severity of NAFLD. Women are protected from the occurrence of CVD and NAFLD [Isidori, Giannetta et al. 2005; Lonardo, Carani et al. 2006]; however, similarly to CVD and atherosclerosis, the estrogen-related hepato-protective effect disappears after menopause [Carulli, Lonardo et al. 2006]. Population-based studies showed that nonalcoholic hepatic steatosis is more common in men than in women; however, following the menopause there is a reversal in gender distribution so that NAFLD is more common in females [Park, Jeon et al. 2006]. In fact, nonalcoholic hepatic steatosis is twice as common in postmenopausal compared to premenopausal women [Hagymasi, Reismann et al. 2009]. It seems that endogenous estrogens play a protective role against the hepatic steatosis. Basic and clinical studies support the hypothesis that estrogens might protect from the development of NAFLD [Carulli, Lonardo et al. 2006; Lonardo, Carani et al. 2006]. For instance, anti-estrogens increase the risk of nonalcoholic steatohepatisis [Bruno, Maisonneuve et al. 2005]. 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 [Suzuki and Abdelmalek 2009]. Therefore, it is logical that several studies indicated that menopause as a natural state of estrogen deficiency is associated with hepatic steatosis [Clark, Brancati et al. 2002; Park, Jeon et al. 2006; Volzke, Schwarz et al. 2007]. The importance of this phenomenon is enlightened by the fact that excessive fat accumulation in liver plays an important role in the development of insulin resistance [Kadowaki, Hara et al. 2003]. Furtheremore, there is a widespread agreement that NAFLD predicts CVD [Bataller, Sancho-Bru et al. 2003]. Increased fat accumulation in the liver is accompanied by atherosclerosis and the metabolic syndrome [Brea, Mosquera et al. 2005; Villanova, Moscatiello et al. 2005; Targher, Bertolini et al. 2006; Targher, Bertolini et al. 2007; Tolman, Fonseca et al. 2007], even independently of intra-abdominal visceral adiposity [Nguyen-Duy, Nichaman et al. 2003; Thamer, Machann et al. 2007]. New findings even indicate that ectopic fat in liver may be more important than visceral fat in characterization

of metabolically benign obesity in humans with which and atherosclerosis has been proposed to exist [Stefan, Kantartzis et al. 2008; Messier, Karelis et al. 2010]. Moreover, Tarantino *et al.* 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" [Tarantino, Pizza et al. 2009]. While it is not completely clear yet, the general association between NAFLD and CVD has just been established by the fact that the liver is involved in regulating/secreting numerous CVD risk factors, notably a 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) [Tarantino, Pizza et al. 2009]. Therefore, due to the increasing prevalence and association with other metabolic disorders in postmenopausal women, it is important that clinicians gain a deep understanding of NAFLD and its clinical presentation as well as therapeutic options in the absence of ovarian secretions. Consequently, information relative to cellular and molecular mechanisms involved in the development of hepatic steatosis in menopausal status has clinical importance. It seems that hormone replacement therapy decreases the risk of steatosis [Hagymasi, Reismann et al. 2009] and the prevalence of NAFLD is lower in postmenopausal women taking hormone replacement therapy than in women not taking it [Clark, Brancati et al. 2002]. Nevertheless, although hormone replacement therapy appears safe in NAFLD, it is not recommended for liver protection because of the increased risk of cardiovascular events [Rossouw, Anderson et al. 2002; McKenzie, Fisher et al. 2006]. A recent review on NAFLD in older women reported 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 [Frith and Newton 2010]; as this is the case for the management of metabolic syndrome.

General mechanisms of adipocyte fat accumulation and hepatic steatosis in rat model of menopause

Among the several endocrine factors that are accountable for the development of obesity, female ovarian hormones have been shown to play a major role [Picard, Deshaies et al. 2000]. Animal models and molecular markers are precious research tools to understand the process leading to adipocyte and liver fat accumulation in a postmenopausal hormonal context. The Ovariectomized (Ovx) rat model of menopause is a model resembling the decline in estrogen levels in postmenopausal women, which is at least partially responsible for the increase in osteoporotic fractures and cardiovascular diseases [Gallo, Zannoni et al. 2005]. In addition, the Ovx rat model may be considered as an experimental model of postmenopausal obesity that may also resemble the characteristic features of a metabolic syndrome occurring in menopause; therefore, Ovx rats can be used as models to reflect the lipid pathogenic changes in perimenopausal or postmenopausal women [Wang, Guo et al. 2004]. This will help to investigate possible lifestyle intervention or new pharmacological treatments.

It is now well established that in animals, Ovx leads to increased food intake and body weight [Latour, Shinoda et al. 2001] thus resulting in increased adipose tissue and liver fat accretion [Deshaies, Dagnault et al. 1997; Picard, Deshaies et al. 2000]. Data from observational and clinical trials evidently show that estrogens possess favorable metabolic effects and estrogen treatment has been shown to decrease body weight gain and fat accumulation in both animals and humans [Tchernof, Calles-Escandon et al. 1998; Seidlova-Wuttke, Hesse et al. 2003; Seidlova-Wuttke, Jarry et al. 2003]. Although hard to separate specifically, estrogens act through two general actions regarding the pathogenesis of Ovx induced fat gain: central effects of estrogens withdrawal (increased food intake and decreased energy expenditure resulting in adipocyte fat gain preferably in intra-abdominal region; since our focus is on hepatic fat accumulation, in our research work we call it ‘‘extra-hepatic effects of estrogens’’) and ectopic effects of estrogens withdrawal (or intra-hepatic: affecting ectopic tissue of liver at molecular level resulting in ectopic fat

accumulation). There might be interactions between two effects of estrogens in terms of adipocyte and ectopic fat accumulation. For example, central effects are indirectly involved in liver fat accumulation via increased fatty acid flow into the liver from circulation (arising from increased food intake and higher intra-abdominal fat depositions).

Mechanisms of estrogen action in brief

It is now well recognized that the effects of estrogens are not limited to the female reproductive system and almost all tissues are under estrogenic influence in both men and women [Ciocca and Roig 1995; Matthews and Gustafsson 2003]. Epidemiological and clinical evidence strongly suggest that estrogens, in particular 17β-estradiol (E2) the most potent and dominant estrogen in mammals, play an important regulatory role in the metabolism and regional distribution of adipose tissue [Wade and Gray 1978; Ohlsson, Hellberg et al. 2000; Mayes and Watson 2004]. Estrogen deficiency leads to increased fat, preferentially in visceral fat, which would link obesity to the susceptibility of related disorders [Pallottini, Bulzomi et al. 2008]. Estrogens promote subcutaneous fat depot after sexual maturation [Ohlsson, Hellberg et al. 2000]. Conversely, in postmenopausal women abdominal fat increases [Sjostrom, Smith et al. 1972]. It seems that E2 controls fat distribution by changing the lipolytic response into the two fat depots differentially, thus favoring fat accumulation in peripheral depots at the expense of the visceral depot [Pallottini, Bulzomi et al. 2008]. Estrogens also regulate activity of lipoprotein lipase (LPL), a major lipogenic enzyme in adipose tissue [Hamosh and Hamosh 1975]. It has been shown in several studies that ovariectomy in female rats results in increased LPL, while estrogen replacement decreased the LPL activity [Mayes and Watson 2004].

Moreover, 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 estrogen effects in terms of different functions including lipid metabolism. The

molecular and biological mechanisms underlying the metabolic actions of estrogen in liver are poorly understood. Estrogen is a steroid hormone mainly produced by ovaries. Its actions are predominantly mediated by genomic mechanisms through its nuclear receptors (ER) α or β [Bjornstrom and Sjoberg 2005]. ERs are ligand-activated transcription factors that mediate estrogens' biological actions in liver and it was shown that ER diminishes sharply at postmenopause [Shimizu 2003; Meza-Munoz, Fajardo et al. 2006]. Outstanding advancements in recent years suggested that estrogens action in vivo is complex and often involves activation of cytoplasmic signaling cascades in addition to genomic actions mediated directly through estrogen receptors α and β; and might simultaneously activate distinct signaling cascades that function as networks to coordinate tissue responses to estrogen [Segars and Driggers 2002]. These orchestrating distinct signaling pathways which involves specific complexes of cytoplasmic proteins might supplement or augment genomic effects of estrogen that are attributable to transcriptional activation by bound receptors [Driggers and Segars 2002]. Therefore, it is not surprising that E2 has been shown to exert rapid non-genomic biological actions through membrane bound subpopulations of ER [Kelly and Levin 2001; Evinger and Levin 2005; Revankar, Cimino et al. 2005]. Interestingly, D'Eon *et al.* reported novel genomic and non-genomic actions of E2 that promote leanness in Ovx animals independently of reduced energy intake [D'Eon, Souza et al. 2005]. Moreover, it has been suggested that E2 reduces adiposity by promoting the use of lipid as fuel which is recognized by the stimulation 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 [Pallottini, Bulzomi et al. 2008].

Central (extra-hepatic) effects of estrogen withdrawal

Obesity results from an imbalance between energy intake and expenditure. Since hyperphagia is a well known response to Ovx and is prevented if estradiol is replaced, many of the effects attributed to estradiol may be explained primarily by changes in food

intake [Richard 1986]. In fact, one view of Ovx-induced obesity is that estrogen removal leads to a marked increase in body energy stores of the rat (via increased energy intake and food efficiency along with decreased energy expenditure), which leads to increased energetic efficiency [Picard, Deshaies et al. 2000; Lemieux, Picard et al. 2003]. This contributes to weight gain, especially as visceral or intra-abdominal fat, that has been reported in both Ovx animals [Paquette, Shinoda et al. 2007] and women during and after menopause [Simkin-Silverman and Wing 2000]. Consequently, determinants of lipid metabolism such as liver triacylglycerol (an index of long-term hepatic lipid accumulation) and adipose tissue lipoprotein lipase activity (the enzyme which hydrolyzes lipoprotein-bound triglycerides and favors tissue uptake of so released fatty acids) are altered in correspondence with increased energy flux [Lemieux, Picard et al. 2003]. 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 [Deshaies, Dagnault et al. 1997]. Therefore, the central effects of estrogen withdrawal indirectly (i.e. via food intake and changes in insulin levels and its efficiency of action) affect liver fat accumulation in Ovx animals [Picard, Deshaies et al. 2000]. Briefly, central effects of estrogen supplementation in Ovx rats have been shown to lower food intake [Gray and Wade 1981; Pedersen, Bruun et al. 2001], decrease adipose tissue lipoprotein lipase (LPL) activity [Gray and Greenwood 1984], increase adipose tissue lipolysis [Darimont, Delansorne et al. 1997], increase spontaneous physical activity [Roy and Wade 1975], and increase energy expenditure [Heine, Taylor et al. 2000; Pedersen, Bruun et al. 2001]. In regard to central effects of estrogen, Picard *et al.* state that Ovx induces obesity by removing the catabolic actions of estrogens, which act upon as yet poorly defined central neuropeptidergic pathways that regulate energy balance [Picard, Deshaies et al. 2000]. For example, estrogen has been reported to have negative effects on 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 [Cooke and Naaz 2004].

Intra-hepatic effects of estrogen withdrawal

Several conditions promote liver TAG accumulation among which an estrogen-deficient state is considerable [Paquette, Shinoda et al. 2007; Barsalani, Pighon et al. 2008; Corriveau, Paquette et al. 2008]. For instance, hepatic steatosis was reported to become evident in an aromatase-deficient mouse (which lacks the intrinsic ability to produce estrogen) and was diminished in animals after treatment with estradiol [Nemoto, Toda et al. 2000]. In addition, visceral obesity, metabolic syndrome with insulin resistance, and hepatic steatosis are the main features of the aromatase knockout (ArKO) mouse phenotype [Simpson, Jones et al. 2005]. Although many of the effects attributed to estrogens in the pathogenesis of Ovx-induced fat gain may be explained primarily by the central effects of estrogens mostly via changes in food intake, D'Eon *et al.* demonstrated that estrogen reduced adiposity in Ovx rodents which is not confounded by differences in food intake [D'Eon, Souza et al. 2005]. Their data are consistent with the phenotypes of both estrogen receptors-α (ERKO) knock-out and aromatase (and thus estrogen)-deficient mice, both of which exhibit increased adiposity with no reported differences in food intake [Heine, Taylor et al. 2000; Jones, Thorburn et al. 2000; Jones, Thorburn et al. 2001; Misso, Murata et al. 2003]. Moreover, the results of Beckett *et al.* suggest that estradiol regulates substrate metabolism in ectopic tissues such as skeletal muscles independent of changes in food intake [Beckett, Tchernof et al. 2002]. Taken together, these data show 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 of diet (amount or type) and energy expenditure [Barsalani, Pighon et al. 2008]. Fisher *et al.* 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 [Fisher, Kohrt et al. 2000]. Likewise, unpublished data from our lab indicate that pair-feeding in Ovx rats does not completely prevent liver fat accretion in rats. Therefore, there must be factors other than food intake in the pathogenesis of liver fat accumulation in estrogen-deficient states.

Some pathways leading to liver lipid infiltration in estrogen deprived states have been investigated. Increased lipid uptake by liver because of increased fatty acid flow from circulation coming from intra-abdominal fat deposition, attributed to the increased food intake after estrogen 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 [Malavazos, Gobbo et al. 2009]. This leads to the accumulation and storage of hepatic fat and the development of hepatic insulin resistance [Xu, Barnes et al. 2003]. However, the portal/fatty acid flux theory has been questioned with the observation that the bulk of portal vein FFAs originate from subcutaneous adipose tissue in overnight-fasted obese individuals [Klein 2004]. Nevertheless, other mechanisms and pathways leading to hepatic steatosis in postmenopausal state need to be considered. Unfortunately, studies on the expression of lipid metabolism-related genes in the liver Ovx rats are limited.

Enhanced uptake mechanisms of lipids by the liver resulting from estrogen deficiency could also play a role yet to be explored. As estrogen levels decline, there may be increased lipogenesis and reduced fatty acid oxidation within the liver [Suzuki and Abdelmalek 2009]. Thus, another possible pathway leading to hepatic steatosis is *de novo* lipogenesis. Liver synthesizes fatty acids *de novo* through a complex cytosolic polymerization in which acetyl-coenzyme A is converted to malonyl-CoA by ACC and undergoes several cycles of metabolic reactions to form one palmitate molecule [Fabbrini, Sullivan et al. 2010]. Liver *de novo* fatty acid synthesis is mostly regulated by three known transcription factors: SREBP-1c, ChREBP, and PPAR-γ [Hashimoto, Cook et al. 2000; Bugianesi, Leone et al. 2002; Matsusue, Haluzik et al. 2003; Evans, Barish et al. 2004]. SREBP1-c activates FAS and SCD-1 genes that are responsible for lipogenesis in the liver [Reddy and Rao 2006]. D'Eon *et al.* investigated the expression of several genes involved in the regulation of lipogenesis in the liver of Ovx-control and Ovx-estrogen (E2) replacement mice [D'Eon, Souza et al. 2005]. Similar to their observations in adipose

tissue, estrogen 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, in another study from our lab, increased lipogenesis in liver of Ovx rats has been reported by changes in the expression and protein levels of lipogenic molecules such as SREBP-1c and SCD-1 [Paquette, Wang et al. 2008]. In addition, suppression of hepatic gene expressions involved in lipid oxidation such as PPAR-α was also reported in these Ovx rats. PPAR-α is a receptor for peroxisome proliferators that functions as a sensor for fatty acids (lipid sensor), and ineffective PPAR-α sensing can lead to reduced energy burning resulting in hepatic steatosis [Reddy and Rao 2006]. Moreover, very recently it has been reported that estrogen removal decreased the rate of fatty acid oxidation by 34% in liver tissue of Ovx rats [Paquette, Chapados et al. 2009]. Furthermore, Na *et al.* reported that estrogen deficiency in the liver of Ovx rats (high-fat fed) raises lipogenesis by increasing mRNA expression of FAS and PPAR-γ, while diminishing lipolysis by decreasing the expression of HSL and PPAR-α mRNAs [Na, Ezaki et al. 2008]. In line with this observation, in a very recent study of Rogers *et al.*, liver from Ovx mice displayed visible steatosis even in a state of pair-feeding that was coincident with a remarkable elevation in hepatic PPAR-γ expression which is known to stimulate a program increasing lipogenic gene expression [Rogers, Perfield et al. 2009]. Accordingly, higher expression of two genes involved in lipogenesis; FAS and ACC was observed in this study. To confirm the role of estrogens in the regulation of hepatic lipid metabolism, it has been shown that 17β-estradiol (E2) 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 [Paquette, Wang et al. 2008; Paquette, Chapados et al. 2009].

Moreover, to our knowledge, the VLDL-TG production and secretion system under low estrogenic condition is not well established. However, very recent data (unpublished) from our laboratory revealed declined VLDL-TG production in Ovx rats [Barsalani, Chapados et al. 2010 submitted paper].

Despite growing number of evidences relating to menopause-associated metabolic disturbances; there are few studies with reference to the underlying pathways of estrogen-deficiency induced hepatic steatosis. Taken together, estrogen withdrawal can have direct effects on hepatocytes and cellular constituents of liver tissue (intra-hepatic effects), as well as central effects on food consumption, energy expenditure, and adipose deposition that contribute to the overall effects on liver fat accretion (Table 4).

Table 4. Effects of estrogen withdrawal on liver fat accumulation.

Central Effects	**Intra-hepatic Effects**
CNS/hypothalamic effects • ↑ Food consumption • ↑ Leptin secretion • ↓ Activity and energy expenditure Lipid profile and adipose tissue effects • Absence of estrogen causes fat redistribution/gain particularly increased intra-abdominal fat and altered lipid homeostasis (portal/fatty acid flux theory)	Lipid uptake • Unknown (possible mechanism of down-regulation of fatty acid uptake via estrogen-dependent pathways, yet to be explored) Lipogenesis • ↑ SREBP-1c • ↑ SCD-1 • ↑ FAS • ↑ ACC • ↑ PPAR-γ Lipid oxidation • ↓ PPAR-α • ↓ HSL • ↓ Fatty acid β-oxidation VLDL-TG production and secretion system • ↓ VLDL-TG production in Ovx rats

Effects of estrogen withdrawal on liver lipid accumulation (hepatic steatosis) may be direct by affecting lipid uptake, *de novo* lipogeneis, lipid oxidation, and liver VLDL-TG production and secretion in liver; or secondary to its effects on the central nervous system (CNS) or fat gain, particularly intra-abdominal (visceral) fat accretion.

Prevention/treatment of adipose tissue and liver fat accumulation in menopausal hormonal state

More than 60% of American postmenopausal women are overweight or obese [Mokdad, Serdula et al. 1999] and as mentioned earlier, it is well established that menopause is associated with weight gain, unfavorable alteration in body composition (elevated visceral fat deposition), and a state of hepatic steatosis [Astrup 1999; Faria, Ribeiro Filho et al. 2002; Volzke, Schwarz et al. 2007]. It seems that hormone replacement therapy (HRT) alleviates these symptoms of menopause [Hassager and Christiansen 1989; Arabi, Garnero et al. 2003; Green, Stanforth et al. 2004]. 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 the evidence of increased risk of heart disease, stroke, venous thromboembolism, and breast cancer with HRT in postmenopausal women [Rossouw, Anderson et al. 2002; Beral 2003]. 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 above two large-scale HRT trials, have led to the conclusion that HRT will not protect future health in postmenopausal women [McPherson 2004; Wegge, Roberts et al. 2004]. Therefore, it seems that the justification for HRT can no longer be applied for disease prevention or treatment, thus 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 [Cassidy 2005].

Interestingly, the most research recommended prevention/treatment for weight gain, elevated visceral fat deposition, and hepatic steatosis is identical which is weight loss through lifestyle interventions including exercise and/or diet. These lifestyle modifications can constitute an important alternative (or complementary) strategy to HRT in postmenopausal women to alleviate concerned disorders. Moreover, very recently Zanesco and Zaros in their review paper reported that in an attempt to reduce the incidence of CVD

in postmenopausal women, a variety of approaches have been used but the results are conflicting and changes in lifestyle have been proposed as a most effective preventive action [Zanesco and Zaros 2009]. It seems there is no substitute for an appropriate lifestyle [Dubnov-Raz, Pines et al. 2007]. Moreover, Hagey and Warren suggested that exercise and nutrition play important roles in the prevention and treatment of obesity, diabetes, and CVD in postmenopausal women [Hagey and Warren 2008]. Data from a 5-year randomized clinical trial known as the Women's Healthy Lifestyle Project, demonstrated that weight gain and increased waist circumference during the peri- to postmenopause can be prevented by a long-term lifestyle dietary and physical activity intervention [Simkin-Silverman, Wing et al. 2003].

One of the most important components of lifestyle relates to physical activity which for a long time has been known to be a powerful low-risk means for the promotion of all aspects of human health including postmenopausal women [Pines and Berry 2007]. Postmenopausal women might demonstrate a greater response to exercise [Hagey and Warren 2008] 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 menopausal women [Rainville and Vaccaro 1984]. Longitudinal and cross-sectional studies have shown that physical activity is associated with lower body fat and less central adiposity in postmenopausal women [Stevenson, Davy et al. 1995; Astrup 1999; Guo, Zeller et al. 1999; Irwin, Yasui et al. 2003; Sternfeld, Wang et al. 2004]. The results of a research by Hagberg *et al.* 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 [Hagberg, Zmuda et al. 2000]. Furthermore, it has been shown that moderate-intensity exercise can result in improvements in coronary/metabolic risk factors such as insulin action in postmenopausal women [Ready, Drinkwater et al. 1995; Ready, Naimark et al. 1996; Asikainen, Miilunpalo et al. 2002; Asikainen, Miilunpalo et al. 2003; Frank, Sorensen et al. 2005]. Given that obesity is extremely prevalent and difficult to treat, prevention of weight gain during this time in a

women's life is an important health goal. A successful model of weight gain prevention has yet to be established [Simkin-Silverman, Wing et al. 2003]. In a cross-sectional study by Hagmar *et al.*, postmenopausal former elite endurance athlete women were investigated in terms of athlete's heart being compared with age-matched sedentary controls. Authors suggested that intense training enhances cardiovascular performance in the aging female athlete [Hagmar, Hirschberg et al. 2005]. This may imply that previous exercise training (during the reproductive period) may be useful in the prevention of deleterious cardio-metabolic effects of menopause. Taken together, it seems that postmenopausal women with high levels of physical activity have lower body fat and abdominal fat and are less likely to gain fat (total and abdominal) during menopause than those with low levels of physical activity [Astrup 1999].

It has been shown that Ovx animal models can benefit from an exercise training program. In 1987, a reduction in fat gain with training has been reported [Richard, Rochon et al. 1987]. Then in 2002, Shinoda *et al.* showed that exercise training has a strong action upon reduction in body fat accumulation following a decrease in estrogen levels [Shinoda, Latour et al. 2002]. 8-wk endurance exercise training in the latter did not reduce overall weight gain and increased food intake brought about by an ovariectomy; suggesting a compensatory increase in muscle weight by training. On the other hand, although food restriction seems to prevent the Ovx-induced weight gain, this treatment suppresses muscle growth in Ovx rats [Fisher, Kohrt et al. 2000]. It appears that increase in body weight and organ weights including muscle mass subsequent to ovariectomy in rats [Booth and Tipton 1969; Santidrian and Thompson 1981] may be a compensatory mechanism to protect the bone loss when the estrogen levels are low. For instance, it has been reported that freely eating Ovx rats suffered less bone loss than did food restricted Ovx animals; suggesting that freely eating Ovx animals were partially protected from bone loss by their greater body weight [Wronski, Schenck et al. 1987]. 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 as there are many studies showing beneficial effects of exercise on

bone tissue in the postmenopausal estrogen-deficient state [Renno, Silveira Gomes et al. 2007].

Another concern of a reduction in estrogenic status is insulin resistance. It is well known that environmental factors such as aging and obesity are linked to the development of a state of insulin resistance and type 2 diabetes mellitus. The prevalence and progression of type 2 diabetes mellitus increase in postmenopausal women that is closely related to estrogen deficiency which leads to higher body weight and fat mass exacerbating insulin resistance [Choi, Jang et al. 2005]. Similarly, several studies have reported insulin resistance in experimental animals after Ovx, which can be reversed by HRT and exercise training, although the results have been somewhat conflicting [Latour, Shinoda et al. 2001; Yakar, Nunez et al. 2006]. In spite of well-documented influences of estrogens on insulin action, the exact mechanisms are not well described [Hansen, McCarthy et al. 1996]. The deterioration of insulin action in Ovx animals might be related to the central effects (i.e. increases in body fat) or to the ectopic effects of estrogen withdrawal (lower estrogen levels per se) or to a combination of both. Ropero *et al.* showed that estrogens in mouse pancreatic β-cells act synergistically with glucose to close K_{ATP} channels through a cGMP-dependent phosphorylation process, consequently resulting in a calcium-mediated stimulation of insulin secretion [Ropero, Fuentes et al. 1999]. This might explain the ectopic and direct effects of estrogen at a molecular level in pancreatic β-cells suggesting that this could also be the case in other ectopic tissues such as muscle and liver.

Exercise training and low-calorie diet have long been prescribed as part of the treatment in the management of type 2 diabetes. There are several studies reporting that food restriction and exercise training increases insulin sensitivity through loss of body fat [Ross, Dagnone et al. 2000; Greco, Mingrone et al. 2002; Shinoda, Latour et al. 2002]. In the study on Ovx diabetic rats, food restriction treatment was partially successful in reversing diabetes progression through body weight and fat reduction (reversing the central effects of estrogen withdrawal) [Choi, Jang et al. 2005]. However, the improvement could not be sustained since this treatment failed to improve pancreatic β-cells function and mass

(showing no ectopic effect of this treatment). On the other hand, regular exercise alone, regardless of estrogen replacement, had the most beneficial effects on insulin sensitivity and β-cell function and mass (reversing both central and ectopic effects of estrogen withdrawal) in Ovx diabetic rats, even though estrogen replacement alone also improved them. Moreover, Saengsirisuwan *et al.*, showed that ovariectomy in female Sprague-Dawley rats results in the development of a systemic metabolic condition representing 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, Pongseeda et al. 2009]. In this study, new evidence was provided showing that whole-body and skeletal muscle insulin resistance is effectively corrected by endurance exercise training alone and estrogen replacement alone. Despite this, similarly to Choi *et al.*, they could find no evidence that exercise training could additively modulate insulin action in Ovx animals that also received estrogen replacement; suggesting that endurance exercise training and estrogen may share common mechanisms to correct defects in ectopic tissues caused by estrogen deficiency [Choi, Jang et al. 2005]. This concept is supported by observations that transcripts encoding estrogen signaling in skeletal muscle, cardiac muscle, and liver are enhanced by regular exercise [Lemoine, Granier et al. 2002; Wiik, Gustafsson et al. 2005; Paquette, Wang et al. 2007].

Resistance training

Weight loss achieved through restrictive diet often results in adverse effects on muscle mass [Villareal, Apovian et al. 2005]. In this regard, resistance training (RT) seems to be a logical choice considering its beneficial effects on muscular strength in postmenopausal women [Bemben, Fetters et al. 2000]. It has been demonstrated that RT exercise can be an effective substitute for hormone replacement therapy in preserving menopause related osteoporosis and sarcopenia [Maddalozzo, Widrick et al. 2007]. In addition to increasing muscle mass and improving muscle function, RT has been reported

to augment resting and total energy expenditure, and to induce decreases in total and abdominal fat [Maesta, Nahas et al. 2007]. On the other hand, there are studies that showed no reduction in fat tissue and Orsatti *et al.* suggest that this discrepancy might be related to the methods used to assess adiposity in postmenopausal women (measurements of skin fold thickness, indirect measurements such as waist circumference and body composition measurements, computerized tomography, dual-energy X-ray absorptiometry) [Orsatti, Nahas et al. 2008]. However, 8 weeks of low intensity, short duration RT program was not sufficient to produce significant alterations in body composition and blood lipid concentrations in postmenopausal women, although it produced substantial improvements in muscle strength [Elliott, Sale et al. 2002]. 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 [Ryan, Pratley et al. 1996]. In these subjects, RT alone or in combination with weight loss program (diet) (RT+WL) improved muscular strength and insulin action and glucose homeostasis. However, the same authors in another study showed that body weight and fat mass did not change with RT alone, but decreased with RT+WL [Ryan, Pratley et al. 2000]. Nevertheless, RT and RT+WL both increased fat-free mass and resting metabolic rate and decreased percent fat in these postmenopausal women [Ryan, Pratley et al. 1995]. Considering the fact that subjects in the RT group were non-obese and subjects in RT+WL group were obese postmenopausal women, this research group suggests that RT may be a valuable component of an integrated weight management program in postmenopausal women. In general, it seems that data from human studies are suggestive of resistance training.

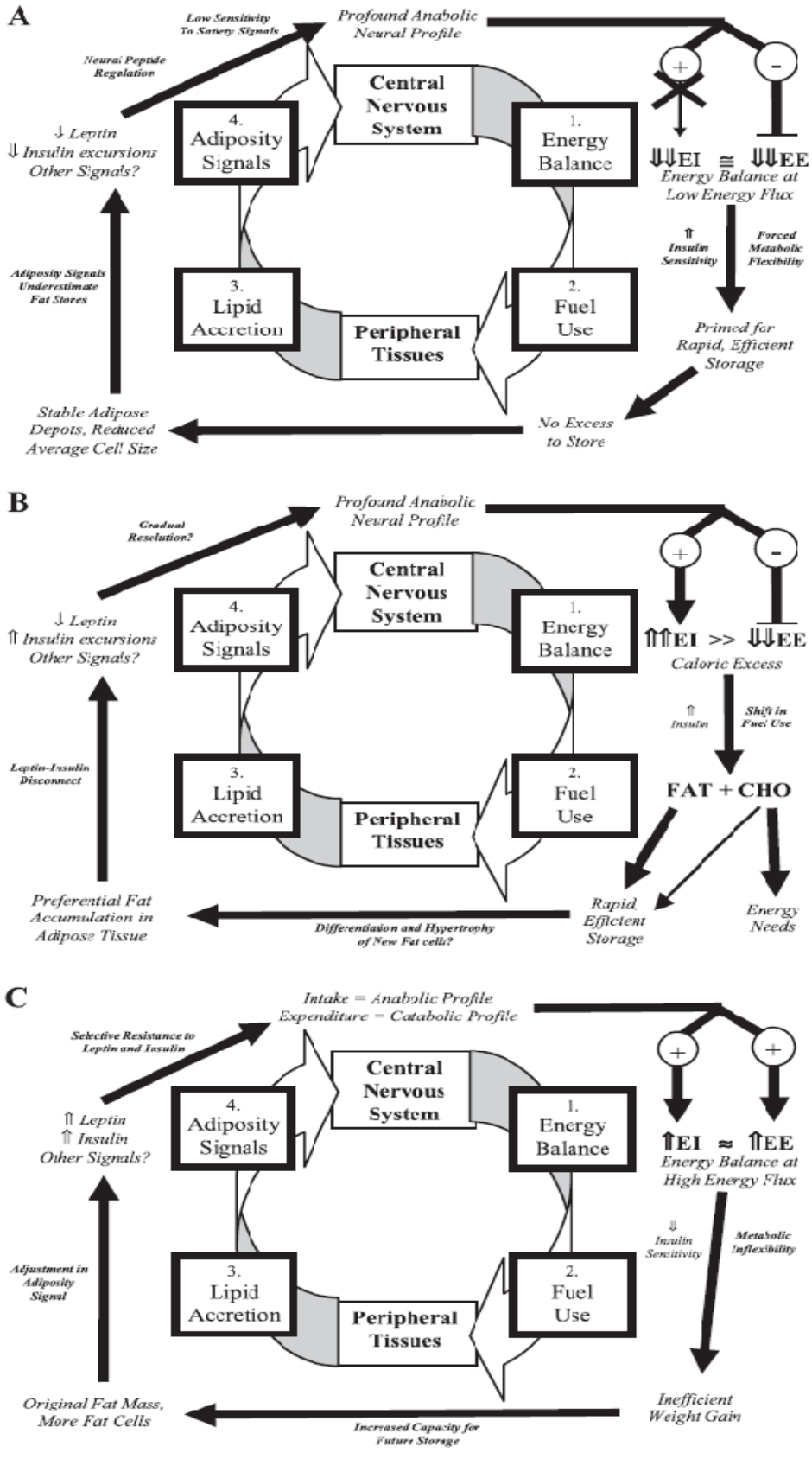
In ovariectomized rats, Corriveau *et al.* have shown that an 8-wk program of resistance training in conjunction with a restrictive diet reduced intra-abdominal fat depot and plasma free fatty acid levels and prevented liver fat accumulation [Corriveau, Paquette et al. 2008]. They concluded that RT is an asset to minimize the deleterious effects of ovarian hormone withdrawal on abdominal fat and liver lipid accumulations in Ovx rats.

Leite *et al.* also recently indicated the potential benefits of resistance training as an alternative strategy to control the negative effects of ovariectomy [Leite, Prestes et al. 2009]. Twelve-wk strength training in Ovx rats decreased fat content in the liver, skeletal muscle, and intra-abdominal adipose tissue and positively changed the lipid profile such as increasing HDL levels while decreasing total cholesterol and LDL levels. In both studies, the RT program consisted of climbing vertical grill with weights attached to the tail of rat. Although these studies did not investigate the mechanism of RT action on liver lipid content, based on evidences on the effects of exercise on AMP-activated protein kinase (AMPK) activation [Griffiths, Baker et al. 1993; Park, Kaushik et al. 2002; Dobrzyn, Dobrzyn et al. 2004; Lavoie and Gauthier 2006], they speculated that RT could induce AMPK activation with a decrease in the expression of transcription factors related to lipogenesis, improving liver fat oxidation and consequently reducing liver lipid content. Moreover, the notion that liver fat follows adipose tissue fat accumulation is always present (exercise has secondary effects on liver fat).

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 strongly supported [Scheen and Luyckx 2002], reductions in weight by dietary restriction are typically modest and are increasingly viewed as an unsustainable outcome of lifestyle modification [Shaw, Gennat et al. 2006; Hansen, Dendale et al. 2007]. It seems that there is a high rate of relapse after diet-induced weight loss [1992]. One of the main underlying problem in this matter appears to be the compensatory metabolic responses to weight reduction which results in a strong drive to regain lost weight [Corbett, Wilterdink et al. 1985; Hill, Thacker et al. 1988; Dulloo and Girardier 1990; Dulloo and Calokatisa 1991; MacLean, Higgins et al. 2004]. Such

responses including enhanced metabolic efficiency with a progressively increasing appetite along with interrelated alterations like improved insulin sensitivity and energetically favorable shifts in fuel utilization characterized by an increased preference for carbohydrate oxidation at the expense of lipid oxidation; may well explain why successful weight reduction is so hard to achieve [MacLean, Higgins et al. 2004]. MacLean *et al.* suggest that these compensatory adjustments in metabolism 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 [MacLean, Higgins et al. 2006]. Their proposed metabolic state after weight-reduction in the context of the homeostatic feedback system that defends fat stores is summarized in Figure 3. It seems that this homeostatic feedback system defending body weight and adiposity is fundamental to the metabolic drive to regain lost weight [Levin and Dunn-Meynell 2000; MacLean, Higgins et al. 2006]. The good thing 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 [Klem, Wing et al. 1997; Wadden, Vogt et al. 1998]. Levin *et al.* reported that regular physical activity lowers the defended level of weight gain and adiposity without a compensatory increase in intake and with a favorable alteration in the development of the hypothalamic pathways controlling energy homeostasis as compared to calorically restricted rats [Levin and Dunn-Meynell 2004; Patterson, Dunn-Meynell et al. 2008]. 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 defense of a lower level of weight gain and adiposity.


A
Low Sensitivity To Satiety Signals
Profound Anabolic Neural Profile
Neural Peptide Regulation
Central Nervous System
4. Adiposity Signals
1. Energy Balance
↓ Leptin
⇓ Insulin excursions
Other Signals?
⇊EI ≅ ⇊EE
Energy Balance at Low Energy Flux
⇑ Insulin Sensitivity
Forced Metabolic Flexibility
Adiposity Signals Underestimate Fat Stores
3. Lipid Accretion
Peripheral Tissues
2. Fuel Use
Primed for Rapid, Efficient Storage
Stable Adipose Depots, Reduced Average Cell Size
No Excess to Store
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Gradual Resolution?
Profound Anabolic Neural Profile
Central Nervous System
4. Adiposity Signals
1. Energy Balance
↓ Leptin
⇑ Insulin excursions
Other Signals?
⇈EI >> ⇊EE
Caloric Excess
⇑ Insulin
Shift in Fuel Use
Leptin-Insulin Disconnect
3. Lipid Accretion
Peripheral Tissues
2. Fuel Use
FAT + CHO
Preferential Fat Accumulation in Adipose Tissue
Differentiation and Hypertrophy of New Fat cells?
Rapid Efficient Storage
Energy Needs
C
Intake = Anabolic Profile
Expenditure = Catabolic Profile
Selective Resistance to Leptin and Insulin
Central Nervous System
4. Adiposity Signals
1. Energy Balance
⇑ Leptin
⇑ Insulin
Other Signals?
⇈EI ≈ ⇈EE
Energy Balance at High Energy Flux
⇓ Insulin Sensitivity
Metabolic Inflexibility
Adjustment in Adiposity Signal
3. Lipid Accretion
Peripheral Tissues
2. Fuel Use
Original Fat Mass, More Fat Cells
Inefficient Weight Gain
Increased Capacity for Future Storage

Figure 3. Taken from [MacLean, Higgins et al. 2006] Metabolic state after weight-reduction (*A*), early in relapse (*B*), and after relapse (*C*). The compensatory adaptations in the periphery, regarding *1*) energy balance, *2*) energetic cost of gain, *3*) lipid accretion, and *4*) adiposity signals that facilitate rapid and efficient weight regain are summarized in the context of the homeostatic feedback system that defends peripheral fat stores. *A*: the metabolic state after weight reduction is diagrammed. The anabolic central nervous system profile promotes a large energy gap with an increased drive to eat and a suppressed expenditure of energy. The weight-reduced state is maintained only if intake is forcefully restricted to the level that energy expenditure is suppressed. Peripheral tissues are forced to be flexible with their use of fuels because of intermittent exogenous fuel availability. Adipose depots are stable, but depleted, and humoral adiposity signals are lower than would be expected for the level of peripheral fat. This blunted signal contributes to the adapted regulatory loop by lowering the sensitivity of neural control centers to hunger and satiety signals in a manner that promotes the overall anabolic output. *B*: with uncontrolled intake, the energy gap is realized. Excess fuels and the accompanying insulin excursions, in the context of insulin sensitive tissues that have the cellular infrastructure to suppress fat oxidation, promote a dramatic shift in fuel utilization whereby exogenous fat is preferentially diverted to storage depots. Ingested energy is rapidly and efficiently diverted toward the repletion of adipose stores. *C*: the pressures driving regain gradually resolve as the lost weight is regained. The metabolic state gradually shifts to one that attempts to prevent further weight gain. The energy gap is minimized at a higher overall energy flux, and peripheral tissues become resistant to insulin's actions and metabolically inflexible. The energetic efficiency of further gain is elevated. The regulatory system achieves a level of equilibrium similar to what is seen before weight loss except that the adipose depots have increased their total capacity to store fat, an effect that may have implications on the continued progression of obesity. In the face of both hyperinsulinemia and hyperleptinemia, the neural control centers exhibit what would appear to be a selective resistance to regulating intake, as both intake and expenditure remain high.

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 above discussion on weight regain, Nicklas *et al.* suggest 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 to regain body weight [Nicklas, Rogus et al. 1997]. They showed that addition of endurance exercise to diet-induced weight loss program minimizes these negative metabolic adaptations in postmenopausal women. Another strategy, substituting a walking training with 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 [Fogelholm, Kukkonen-Harjula et al. 2000]. However, randomized controlled trials comparing different strategies such as diet and diet plus exercise in postmenopausal women are few and the results do not allow a firm conclusion as to whether physical activity may prevent or limit the gain of total and abdominal fat during menopause or whether it may be effective as part of an obesity treatment program [Astrup 1999].

The specific case of liver fat accumulation and exercise training

A fatty liver overproduces components of the metabolic syndrome (dyslipidemia, hyperglycemia) and new data imply that fat accumulation in the liver is a key player in the pathogenesis of insulin resistance and the metabolic syndrome which may distinguish who develop the syndrome from those who do not, even independently of obesity [Kotronen, Westerbacka et al. 2007; Kotronen, Seppala-Lindroos et al. 2008]. Despite growing evidence of the metabolic and cardiovascular impact of liver lipid infiltration, there are relatively few studies examining the effects of lifestyle intervention [Johnson, Sachinwalla et al. 2009]. Interventions which reduce hepatic triglyceride concentrations are often come

with significant improvements in metabolic function such as insulin resistance [Petersen, Dufour et al. 2005]. On the other hand, there is increasing evidence suggesting that physical activity and increased fitness can improve the metabolic syndrome even without weight loss [Ross, Dagnone et al. 2000; Cox, Burke et al. 2004; Ross, Janssen et al. 2004; Nassis, Papantakou et al. 2005]. Very recent experimental study showed that short-term 4-week aerobic exercise training results in a significant reduction in both hepatic lipids and visceral adipose tissue even in the absence of body weight reduction [Johnson, Sachinwalla et al. 2009]. Moreover, concurrent exercise training in animals prevented high-fat-diet-induced intra-abdominal fat accumulation and hepatic steatosis [Gauthier, Couturier et al. 2003]. Therefore, it is very relevant to investigate the effects of exercise training in estrogen-deficient states which is characterized with increased visceral adipose tissue and hepatic lipid accumulation.

Pathogenic role of sub-acute systemic inflammation in obesity and insulin resistance

Inflammation

Inflammation is one of the first responses of the immune system to infection or irritation. It is stimulated by chemical factors, including specialized chemical mediators, called cytokines and ranges from a local to a systemic response to cellular injury [Moldoveanu, Shephard et al. 2001]. The local response to infections or tissue injury involves the production of cytokines that are released at the site of inflammation. Cytokines are small polypeptides, which were originally discovered to have immunoregulatory roles [Akira and Kishimoto 1992; Akira, Taga et al. 1993]. Some of these cytokines facilitate an influx of lymphocytes, neutrophils, monocytes, and other cells. The local inflammatory

response is accompanied by a systemic response known as the acute-phase response. This response includes the production of a large number of hepatocyte-derived acute phase proteins, such as C-reactive protein (CRP). The initial cytokines in the cytokine cascade are (named in order) tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, IL-1 receptor antagonist (IL-1ra), and soluble TNF-α receptors (sTNF-R) [Petersen and Pedersen 2005]. IL-1ra inhibits IL-1 signal transduction and sTNF-R represents the naturally occurring inhibitors of TNF-α [Dinarello 1991; Akira and Kishimoto 1992; Akira, Taga et al. 1993]. In response to an acute infection or trauma, the cytokines and cytokine inhibitors may increase several fold and decrease when the infection or trauma is healed. When inflammation overwhelms the whole organism, systemic inflammatory response syndrome (SIRS) is diagnosed. When it is due to infection, the term sepsis is applied.

With the discovery of interleukins, another concept of systemic inflammation was developed. Although the processes involved are identical, this form of inflammation is not confined to a particular tissue but involves the endothelium (lining of blood vessels) and many other organ systems. During the last decade, it has become clear that inflammatory mechanisms are key players in pathological processes of several chronic diseases such as ischemic cardiovascular disease (CVD), insulin resistance, type 2 diabetes (T2D), colorectal cancer, stroke, chronic obstructive pulmonary disease, and Alzheimer's disease; which are among the most common causes of mortality in the Western world [Bruunsgaard 2005]. At the same time, it has been recognized that cytokines are not only important signals in immune function, as they also represent important regulators of endocrine systems, the metabolism, the coagulation system, and the brain function [Febbraio and Pedersen 2002]. In addition, it has been discovered recently that circulating levels of cytokines in vivo are affected significantly by contributions of cells outside the immune system such as adipose tissue, skeletal muscle, and endothelial cells in healthy humans; e.g., 30% of IL-6 in plasma is derived from fat tissue [Mohamed-Ali, Goodrick et al. 1997]. The concept of regulatory adipokines has developed together with the discovery of fat tissue as an important endocrine organ, producing and secreting classical cytokines

including TNF-α, IL-6, IL-18, as well as a wide range of other new peptides [Kershaw and Flier 2004]. Moreover, it was demonstrated that working skeletal muscles produce and also release cytokines to the circulation [Febbraio and Pedersen 2002]. Therefore, cytokine production is not restricted to the context of infections.

Systemic low-level inflammation is defined as two- to fourfold elevation in circulating levels of cytokines, natural occurring cytokine antagonists, and acute-phase proteins, as well as minor increases in counts of neutrophils and natural killer cells [Bruunsgaard and Pedersen 2003]. Although these increases are far from levels observed during acute/severe infections, systemic low-level inflammation is strongly associated with lifestyle factors such as obesity, and dietary patterns [Bermudez, Rifai et al. 2002; Esposito, Marfella et al. 2004]. Chronic low-grade inflammation accompanies aging as well as some chronic medical disorders and it is a strong, consistent, and independent predictor of all-cause mortality and CVD-cause mortality in elderly populations [Harris, Ferrucci et al. 1999; Yeh, Hafner et al. 2004]. Several reports investigating various markers of inflammation in different population groups have confirmed an association between low-grade systemic inflammation on one hand and the metabolic syndrome, Type 2 diabetes, and atherosclerosis on the other [Barzilay, Abraham et al. 2001; Ford 2002; Duncan, Schmidt et al. 2003]. It seems that systemic low-grade inflammation is a cause as well as a consequence of pathological processes [Bruunsgaard 2005].

Circulating inflammatory markers in metabolic syndrome

In sepsis and experimental models of sepsis (the processes involved are identical as in low-level systemic inflammation), the cytokine cascade consists of TNF-α, IL-1β, IL-6, IL-1ra, sTNF-R, and IL-10 [Akira, Taga et al. 1993]. The first two cytokines in the cytokine cascade, that is, TNF-α and IL-1β, along with IL-2, IL-8, and IL-15 are usually referred to as pro-inflammatory cytokines [Moldoveanu, Shephard et al. 2001]. While IL-1ra, sTNF-R, IL-4, IL-10 and IL-13 are well-known anti-inflammatory cytokines

[Moldoveanu, Shephard et al. 2001]. TNF-α and IL-1 stimulate the production of IL-6, which has been classified as both a pro- and anti-inflammatory cytokine [Tilg, Dinarello et al. 1997] depending on the origin of its production such as adipose and liver tissue in obesity-originated versus muscles during exercise, respectively. IL-10 attenuates the cell surface expression of TNFRs and it inhibits the production of cytokines by monocytes and type 1 T cells [Kolling, Hansen et al. 2001]. However, circulating levels of cytokines work in a network, and their levels are found to inter-correlate, e.g., plasma levels of TNF-α were positively correlated with IL-6, sTNF-R, and CRP in centenarians [Bruunsgaard 2005; Petersen and Pedersen 2005].

Cytokines such as IL-6, IL-1β, and TNF-α which are produced during and participate in inflammatory processes, are the chief stimulators of the production of acute-phase proteins [Gabay and Kushner 1999]. The acute phase reaction is a nonspecific physiological response to tissue injury, infection, inflammation, and disease activity; whose functions include repairing tissue damage, containing infections, promoting wound healing and triggering host defense mechanisms such as the innate immune response [Chen 2006; Kalani, Judge et al. 2006]. As mentioned, this response is carried out by secretion of acute phase proteins coming mainly from hepatocytes including CRP, serum amyloid A (SAA), fibrinogen, and plasminogen activator inhibitor-1 (PAI-1) [Gabay and Kushner 1999]. Among the acute phase markers, CRP that functions primarily to recognize and eliminate pathogens and damaged cells by activating the complement system and phagocyte cells [Volanakis 2001], is considered as an excellent pro-inflammatory biomarker, and received more attention due to its potential in predicting cardiovascular disease in several populations including postmenopausal women [Tracy, Lemaitre et al. 1997; Ridker, Hennekens et al. 2000]. Plasma CRP concentrations have also been reported to increase in the metabolic syndrome and diabetes [Danesh, Whincup et al. 2000]. Moreover, IL-6, another acute phase cytokine and the main stimulator of liver CRP production, has been implicated in developing the inflammatory response persistent in cardiovascular disease [Ridker, Cushman et al. 1997]. Elevated levels of circulating IL-6 have been associated

with several disorders and plasma concentrations of IL-6 and TNF-α have been shown to predict the risk of myocardial infarction in several studies [Ridker, Rifai et al. 2000]. It must be mentioned that although IL-6 is generally regarded as a pro-inflammatory cytokine, it can play a contrary role. For example, it is now well known that IL-6 is rapidly released into the circulation following exercise [Febbraio and Pedersen 2002] and it seems that when IL-6 is produced by working muscles independently of TNF-α/IL-1β, it exerts mainly anti-inflammatory activities [Bruunsgaard 2005]. Furthermore, other cytokines originating from adipocytes or other cells including TNF-α are also involved in this process [Maachi, Pieroni et al. 2004]. TNF-α is a pro-inflammatory cytokine produced by different tissues such as immune cells and adipocytes and it has been shown to play a role in obesity-induced local and systemic insulin resistance [Bastard, Maachi et al. 2006]. There is accumulating data to suggest that TNF-α plays a direct role in the metabolic syndrome. TNF-α, together with IL-6 and other chemokines, mediates macrophage infiltration that causes adipose tissue inflammation in obesity leading to deregulated secretion of adipokines and insulin resistance [Schenk, Saberi et al. 2008]. Consequently, it is not surprising that patients with diabetes demonstrate high expression of TNF-α in plasma [Winkler, Salamon et al. 1998; Mishima, Kuyama et al. 2001] and in skeletal muscle [Saghizadeh, Ong et al. 1996], and it is likely that adipose tissue is the main source of the circulating TNF-α [Coppack 2001].

Taken as a whole, Esposito and Giugliano report that elevated circulating levels of major pro-inflammatory cytokines including CRP, IL-6, TNF-α, and IL-1β along with low levels of anti-inflammatory cytokines such as IL-10 are associated with the metabolic syndrome [Esposito and Giugliano 2004].

Cellular inflammatory responses in obesity and insulin resistance

Weight gain and obesity are most important risk factors for diseases ranging from insulin resistance and T2DM to atherosclerosis and the sequelae of NAFLD [Shoelson,

Herrero et al. 2007]. In recent years, it has become clear that metabolic disturbances related to fat accumulation in adipocytes and ectopic tissues such as the liver, are associated to sub-clinical inflammation [Esposito and Giugliano 2004; Bruunsgaard 2005]. Obesity is strongly associated with enhanced circulating TNF-α levels, whereas weight loss reduces systemic levels [Dandona, Weinstock et al. 1998]. Adipose tissue from obese individuals shows accumulation of macrophages, which provide the major cellular source of a concomitant, enhanced, local expression of the TNF-α and IL-6 proteins [Weisberg, McCann et al. 2003]. In fact, obesity is associated with accretion of lipids into adipocytes and expansion of adipose tissue which produce pro-inflammatory cytokines like TNF-α, IL-6, resistin, MCP-1, and PAI-1; initiating an inflammatory signal that in turn, results in a state of local and systemic insulin resistance (Figure 4) [Shoelson, Herrero et al. 2007].

A model on how inflammation leads to insulin resistance has been proposed by Chen that is illustrated in Figure 5 [Chen 2006]. It seems that adipokines such as TNF-α induces insulin resistance in experimental animal models by mechanisms that involve serine phosphorylation of the insulin receptor substrate 1 (IRS-1) [Hotamisligil, Peraldi et al. 1996]. This phosphorylation reduces insulin receptor tyrosine kinase activity in response to insulin and the ability of IRS-1 to associate with the insulin receptor and thereby interfering with downstream insulin signal transduction [Dandona, Aljada et al. 2005]. The responsible intracellular pathways involve activation of c-Jun N-terminal kinases (JNKs/stress activated protein kinases) [Hirosumi, Tuncman et al. 2002] and the inhibitor of κB kinase (IKKβ) [Arkan, Hevener et al. 2005] whereas activation of members in the suppressor of cytokine signaling (SOCS) family represents alternative intracellular stress pathways in cytokine-mediated inhibition of insulin signaling [Ueki, Kondo et al. 2004]. IKKβ activates nuclear factor of kappa B (NF-kB), a master regulator of inflammation and transcription factor that stimulates production of many inflammatory markers. It seems that IKKβ/NF-kB signaling pathway which is activated by pro-inflammatory cytokines is a key pathway in tissue inflammation [Chen 2006]. Increased free fatty acids (FFA) levels have

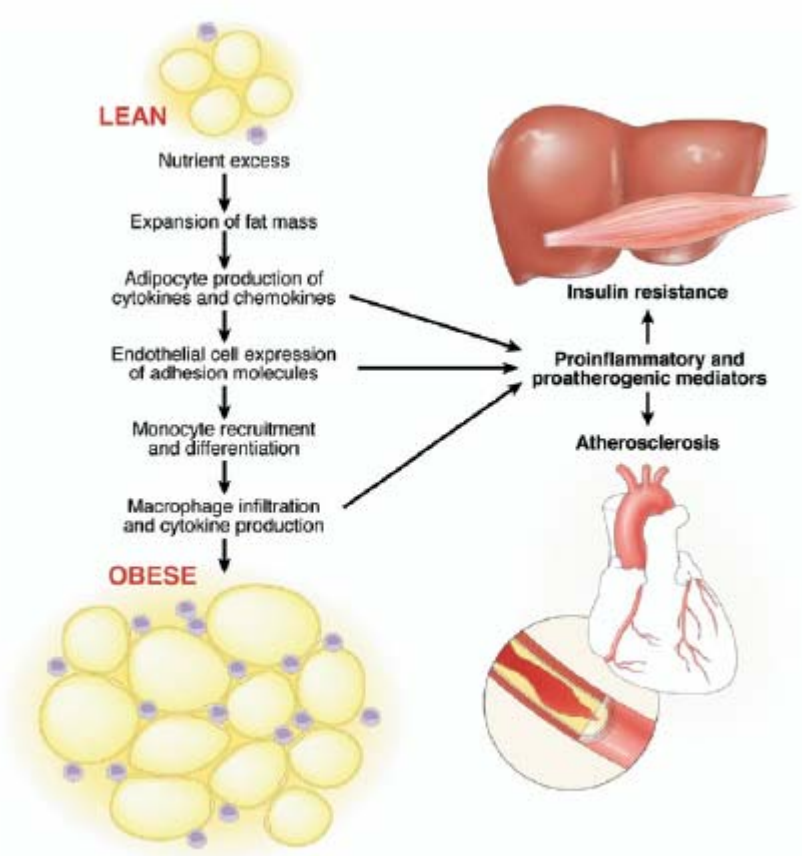

Figure 4. Taken from [Shoelson, Herrero et al. 2007] Potential mechanisms for obesity-induced adipocyte inflammation. The accumulation of lipids in adipose tissue and the expansion of the fat mass lead to the initiation of an inflammatory process. This may be initiated through the production of pro-inflammatory cytokines and chemokines by the adipocytes, including TNF-α, IL-6, leptin, resistin, MCP-1, and PAI-1. Endothelial cells respond through the increased expression of adhesion molecules, which along with the chemokines serve to recruit immune cells including monocyte-derived macrophages to the adipose tissue. Together, the adipocyte-, immune cell-, and endothelial cell-derived substances create an inflammatory milieu that promotes insulin resistance locally. Similar pro-inflammatory and pro-atherogenic mediators enter the circulation to promote insulin resistance and increase the risk for atherosclerosis.

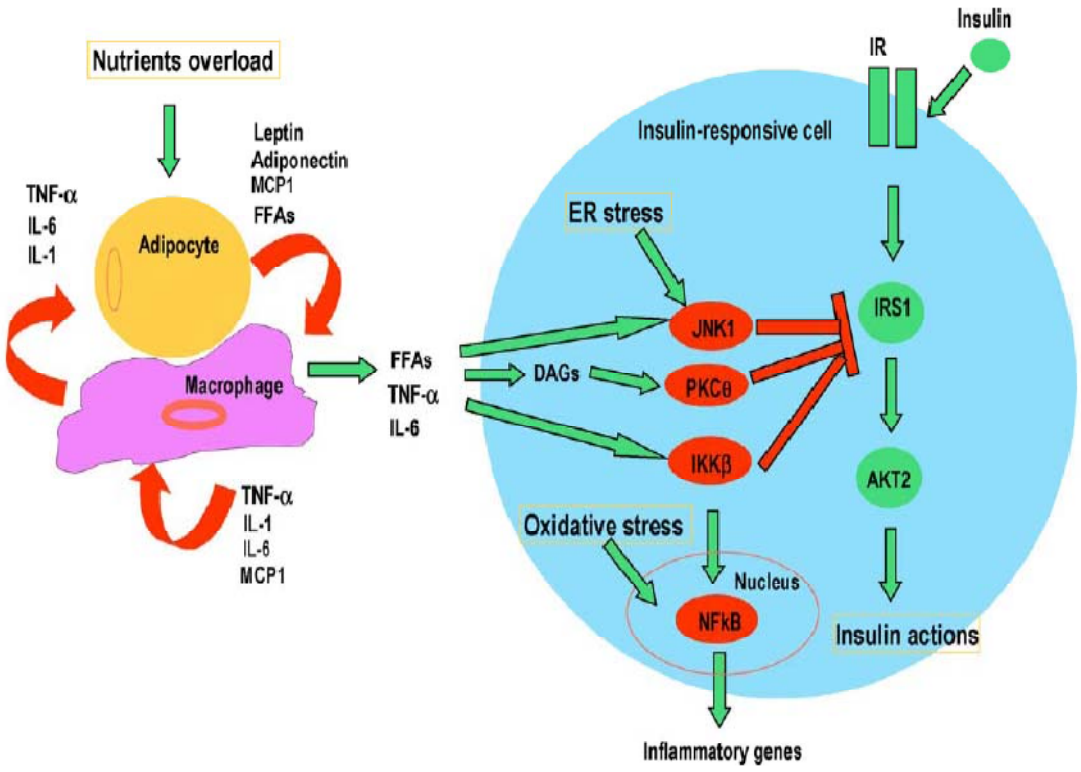

Figure 5. Taken from [Chen 2006] Hypothetical model of metabolic stress, cellular inflammatory responses and effects on insulin signaling pathway. Nutrient overload in adipose tissue leads to increased secretion of FFAs and pro-inflammatory adipokines, which activate resident macrophages. Activated macrophages in turn secrete cytokines and chemokines to recruit additional monocytes and macrophages into fat. The secreted FFAs, IL-6 and TNF-α cause insulin resistance in adipocytes through activation of several serine-threonine kinase pathways that interfere with insulin signaling via serine phosphorylation and subsequent inactivation of IRS-1. In addition, IKKβ and JNK1 activate transcription factors which increase expression of cytokines and inflammatory genes. This vicious cycle amplifies inflammation and accentuates insulin action. Obesity-mediated metabolic stress also adds burden to cellular machineries, leading to ER stress or mitochondria oxidative stress, which again activate IKKβ/NF-kB and JNK1 inflammatory pathways. These same immune modulators similarly lead to impaired insulin action in other tissues like liver and muscle. FFAs: free fatty acids; DAG: diacylglycerol; ER: endoplasmic reticulum.

also been implicated as a causative factor in phosphorylation of IRS-1 [Dandona, Aljada et al. 2005] through increased intra-cellular fatty acyle-CoA and diacylglaycerol (DAG) concentrations and activation of pro-inflammatory kinase, protein kinase C-θ (PKC-θ) [Gao, Zhang et al. 2004]. Moreover, it has been shown that nutritional fatty acids, whose circulating levels are often increased in obesity, activate toll-like receptors (critical players of innate immune system) which in turn trigger pro-inflammatory pathways and induce cytokine expression in a variety of cell types [Shi, Kokoeva et al. 2006]. On the other hand, obesity-mediated metabolic stress (increased fatty acids and glucose metabolism and reactive oxygen species) also exerts a higher-than-normal load to cellular machineries leading to endoplasmic reticulum (ER) stress or mitochondria oxidative stress, which activate both IKKβ/NF-kB and JNK1 inflammatory pathways [Furukawa, Fujita et al. 2004; Ozcan, Cao et al. 2004; Summers 2006].

Moreover, inflammatory markers and mediators such as CRP and IL-6 are synthesized in the liver, suggesting hepatic low-level inflammation most likely secondary to hepatic steatosis, might be involved in the development of insulin resistance and the metabolic syndrome. The findings of Cai *et al.* demonstrate that fat accumulation in the liver leads to sub-acute hepatic inflammation via IKKβ/NF-kB activation and downstream pro-inflammatory cytokine production including IL-6, TNF-α, and IL-1β [Cai, Yuan et al. 2005] . This suggests that hepatocyte lipid accumulation (steatosis) might induce a sub-acute inflammatory response in liver that is similar to adipose tissue inflammation. Alternatively, pro-inflammatory substances in the portal circulation, potentially produced in abdominal fat, might initiate hepatic inflammation [Shoelson, Lee et al. 2006]. In any case, it seems that NF-κB pathway is stimulated in hepatocytes, and cytokines including IL-6, TNF-α, and IL-1β, are overproduced in a fatty liver. The pro-inflammatory cytokines participate in the development of insulin resistance both locally in liver and systemically [Cai, Yuan et al. 2005].

According to Shoelson *et al.*, skeletal muscle is another major site of insulin resistance and a target of inflammation-induced insulin resistance as opposed to a site of

initiation (Figure 6) [Shoelson, Lee et al. 2006]. However, in contrast with this, a study by Boden *et al.* [Boden 2006] indicates that elevated plasma free fatty acid levels, either as a result of obesity or high-fat diet feeding, produces low-grade inflammation in skeletal muscle and liver through activation of NF-κB, as in adipose tissue, resulting in the releasing several pro-inflammatory and pro-atherogenic cytokines leading to insulin resistance. Finally, vascular inflammation is central in the pathology of atherosclerosis [Ross 1999] and inflammation (in adipose tissue and liver) might be a connecting link between obesity and many of its pathological metabolic disorders such as atherosclerosis by infiltrating the artery wall [Surmi and Hasty 2010] (Figure 6). Apart from the underlying mechanisms, the pro-inflammatory state that accompanies the metabolic syndrome associates with both insulin resistance and endothelial dysfunction, providing a connection between inflammation and metabolic processes which is highly deleterious for vascular functions [Esposito and Giugliano 2004].

Physical activity and systemic low-level inflammation

A recent number of papers have documented that self-reported physical activity or physical performance is correlated inversely with systemic low-level inflammation, although the lack of an association has also been reported (Table 5) [Bruunsgaard 2005]. However, the findings of cross-sectional studies demonstrating an association between physical inactivity and low-grade systemic inflammation in healthy subjects and in elderly people [McFarlin, Flynn et al. 2006] [King, Carek et al. 2003; McFarlin, Flynn et al. 2006] together with the results of longitudinal and prospective studies showing that regular training reduces CRP level, suggest that physical activity as such may suppress systemic low-grade inflammation [Mattusch, Dufaux et al. 2000; Kasapis and Thompson 2005]. Accordingly, a high level of physical activity has been shown to be associated with reduced levels of inflammatory mediators in the range of 20–60% compared with a sedentary lifestyle among apparently healthy middle-aged and older US

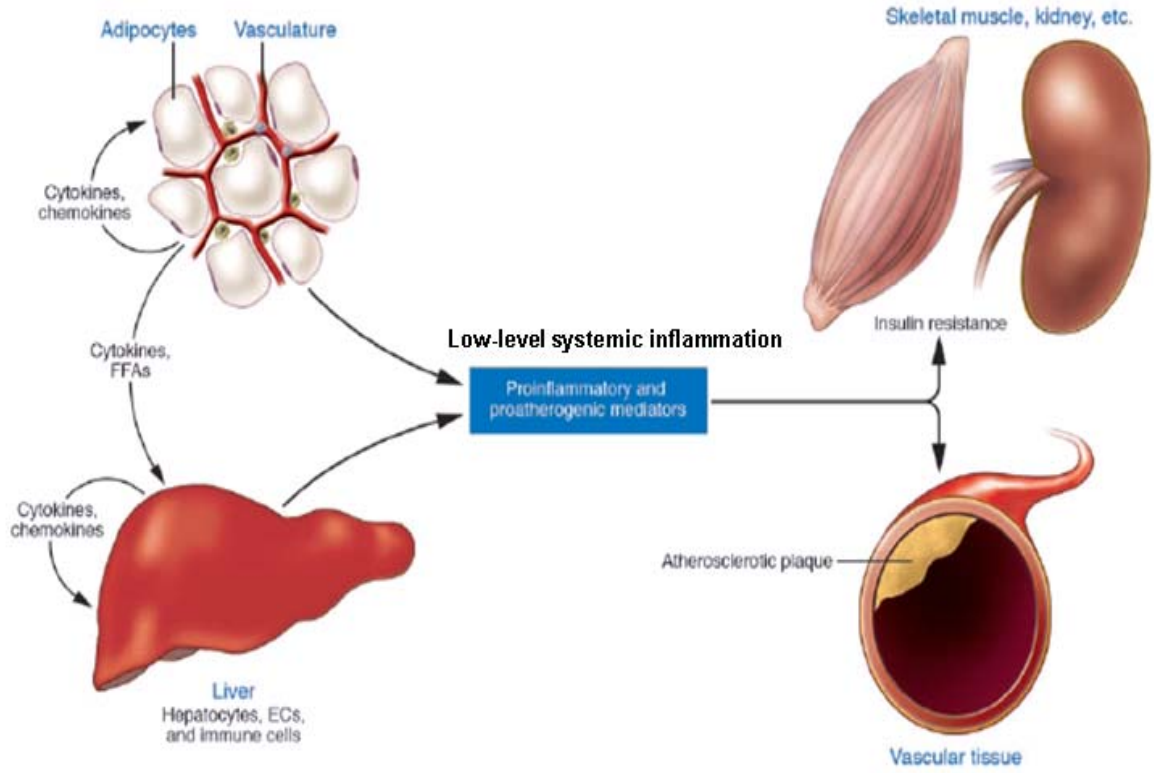

Figure 6. Taken from [Shoelson, Lee et al. 2006] Local, portal, and systemic effects of inflammation in insulin resistance and atherogenesis. Increasing adiposity activates inflammatory responses in fat and liver, with associated increases in the production of cytokines and chemokines. Immune cells including monocytes and macrophages are recruited and/or activated, and together, they cause local insulin resistance. Hepatic steatosis and/or portal delivery of abdominal fat–derived cytokines and lipids contribute to hepatic inflammation and insulin resistance. Pro-inflammatory and pro-atherogenic mediators are produced in adipose tissue and liver as well as associated immune cells. This creates a systemic inflammatory diathesis that promotes insulin resistance in skeletal muscle and other tissues and atherogenesis in the vasculature.

Table 5. Taken from [Bruunsgaard 2005] Self-reported physical activity and physical performance in relation to low-level inflammation in epidemiological studies

Subjects	Circulating inflammatory parameters	High physical activity versus sedentary lifestyle
3042 adults (>18 years) without CVD	TNF, IL-6, CRP, SAA, WBC, fibrinogen	Lower levels of all parameters
Same population as in ref. [97] divided into 701 adults with the metabolic syndrome and 2341 without	TNF, IL-6, CRP, SAA, WBC, fibrinogen	High physical activity in both groups were associated with a lower degree of inflammation
3075 well-functioning humans aged 70–79 years	TNF-α, IL-6, and CRP	Lower levels of TNF-α and IL-6
892 male subjects, free from clinical CVD aged 35–59 years	CRP, SAA, fibrinogen	CRP and fibrinogen reduced in univariate analyses but not in multivariate analyses adjusting for BMI, smoking, education, diabetes, lipids, alcohol
1020 humans aged >65 years living in the area of Chianti	CRP, IL-6, TNF-α, IL-10, IL-1β, IL-6sR, and IL-1Ra	CRP, IL-6, and IL-1Ra were correlated inversely with physical performance and hand-grip strength, independently of demographics, chronic conditions, medication use, and other biological variables
760 humans aged 49–70 years with plaque in carotid artery without symptoms	CRP	No difference
114 postmenopausal women	CRP	Reduced CRP
67 male ultramarathon runners and 63 controls matched by sex, age, and BMI	IL-6, CRP	Reduced CRP but not IL-6 independently of leptin and BMI
109 men and women aged 20–70 years	CRP	No association. CRP was associated with BMI
333 relatively healthy 80-year-old humans	TNF-α and IL-6	Lower levels of TNF-α and IL-6
4072 adults >17 years	CRP, fibrinogen, WBC	Reduced levels of at least one parameter
133 postmenopausal women aged 50–73 years without CVD or diabetes	CRP	Reduced CRP independent of oral HRT use, age, smoking, alcohol consumption, aspirin, and statin but not when body fat and age were included in the multivariate analysis
870 healthy persons aged 70–79 years	IL-6 and CRP	High levels of recreational activity associated with reduced levels of IL-6 and CRP
12 healthy men aged 65–75 years; 6 active and 6 less active	MIP-1α, IL-1Ra, IL-1β, IL-6, IL-10, and CRP	Reduced IL-6 and increased IL-10
356 male and 103 female athletes, 45 male and 40 female untrained controls, and 35 elderly coronary patients	CRP	Reduced CRP in swimmers and rowers compared with untrained controls

SAA, Serum amyloid A; WBC, white blood cell count; HRT, hormone replacement therapy; MIP-1α, macrophage-inflammatory protein-1α; circulating levels, concentration in serum or plasma.

adults [Abramson and Vaccarino 2002]. In addition, several studies have reported that exercise intervention programs reduce systemic low-level inflammation in patients with coronary heart disease [Oberbach, Tonjes et al. 2006], claudicants [Tisi, Hulse et al. 1997], patients with chronic heart failure [Gielen, Adams et al. 2003], and also in healthy young adults [Mattusch, Dufaux et al. 2000]. Improved measures of chronic inflammation markers with exercise training in animal models has also been reported [Kalani, Judge et al. 2006; de Lemos, Reis et al. 2007].

Several studies have shown an anti-inflammatory effect of acute physical exercise, characterized through increased circulating concentrations of IL-10, IL-1 receptor antagonist (IL-1ra), soluble receptor of TNF (TNFRs) [Petersen and Pedersen 2005]. It seems that the cytokine response to exercise differs from that elicited by severe infections [Febbraio and Pedersen 2002]. An acute bout of exercise appears to mediate anti-inflammatory effects in skeletal muscle and fat tissue [Ostrowski, Rohde et al. 1999; Febbraio and Pedersen 2002; Febbraio, Hiscock et al. 2004; Kelly, Keller et al. 2004; Petersen and Pedersen 2005]. The underlying mechanisms appear to involve IL-6-dependent [Petersen and Pedersen 2005] and –independent [van der Poll, Coyle et al. 1996; Kolling, Hansen et al. 2001; Keller, Keller et al. 2004] pathways. Muscle contractions induce a myokine response characterized by a large release of IL-6 from working muscles, independently of pro-inflammatory markers such as TNF-α; which is followed by elevations in circulating levels of well-known anti-inflammatory cytokines and cytokine inhibitors including IL-1ra, IL-10, and sTNFRs [Ostrowski, Rohde et al. 1999]. It has been suggested that the contraction-induced IL-6 expression in skeletal muscle is a specific biochemical phenomenon with the purpose of mobilizing substrate from fuel depots within the body to facilitate energy metabolism [Pedersen, Steensberg et al. 2003]. According to Pederson's theory, contracting skeletal muscles release myokines, which work in a hormone-like fashion, exerting specific endocrine effects on visceral fat and other ectopic fat deposits while other myokines will work locally within the muscle via paracrine mechanisms, exerting their effects on signaling pathways involved in fat oxidation

[Pedersen 2009]. This may provide a conceptual basis to understand the mechanisms whereby exercise influences metabolism and exerts anti-inflammatory effects; playing an important role in the health-beneficial effects of exercise. For instance, even moderate physical activity is probably sufficient to induce the anti-inflammatory effects of exercise, as an increased transcription rate of the IL-6 gene is already detected after 30 min of two-leg extensor exercise at 60% of the individual maximal power output [Keller, Steensberg et al. 2001]. Thus, only 30 min of moderate exercise on a regular basis probably has the power to facilitate an anti-inflammatory environment characterized by enhanced levels of IL-10, IL-1Ra, and sTNFRs between bouts of physical activity [Bruunsgaard 2005]. Although the exact link between acute and long-term effects yet to be known, regular exercise protects against diseases associated with chronic systemic low-grade inflammation; and this long-term effect might be ascribed to the anti-inflammatory response elicited by an acute bout of exercise [Petersen and Pedersen 2005]. In theory, a reduced local pro-inflammatory markers production could explain a part of the decline in systemic low-level inflammation together with the improvement in symptoms and risk factors (mainly decreased visceral and ectopic fat accumulation) associated with the metabolic syndrome, CVD, and T2DM in relation to regular exercise [Bruunsgaard 2005].

The importance of inflammatory cytokines in fatty liver and insulin resistance

Obesity is associated with a chronic inflammatory response characterized by abnormal cytokine production and activation of inflammatory signaling pathways, which has been proposed to play a key role in the pathogenesis of obesity-related insulin resistance and NAFLD [Hotamisligil 2006]. The contribution of the adipose tissue versus the liver as the major sources of circulating and systemically active pro-inflammatory cytokines in these diseases remains unclear. However, once obesity leads to fatty liver, mostly observed in cases of obesity, the liver itself can become a major source of various

inflammatory mediators [Tilg and Moschen 2008; Fabbrini, Sullivan et al. 2010]. In fact, many inflammatory markers that have been associated with insulin resistance, T2DM, metabolic syndrome, and cardiovascular diseases such as CRP, PAI-1, and fibrinogen are liver proteins [Cai, Yuan et al. 2005]. Bruce and Byrne suggest that hepatic ectopic fat accumulation per se is associated with a pro-inflammatory state; together being central to the development of the metabolic syndrome [Bruce and Byrne 2009]. Accordingly, it has been shown that hepatic steatosis increased production of pro-inflammatory cytokines including IL-6 [Wieckowska, Papouchado et al. 2008], showing the strong link between NAFLD and increased cardiovascular risk [Alkhouri, Tamimi et al. 2009]. Similarly, in animal models, high-fat diet and genetically induced obesity resulted in hepatic steatosis, insulin resistance, and increased hepatic pro-inflammatory markers including NF-kB activity, IL-6, IL-1β, TNF-α [Samuel, Liu et al. 2004; Cai, Yuan et al. 2005]. Joshi-Brave *et al.* induced lipid accumulation in hepatocytes (rat and human primary hepatocytes) by exposing them to pathophysiologically relevant concentrations of palmitic acid to stimulate the excessive influx of fatty acids into hepatocytes [Joshi-Barve, Barve et al. 2007]. This was accompanied by hepatic steatosis (increased intracellular TAG) that induced hepatic inflammation (elevated pro-inflammatory IL-8) through activation of NF-kB and JNK1. Moreover, Cai and colleagues demonstrated that sub-acute hepatocellular activation of NF-kB caused hepatic inflammation without steatosis and resulted in both hepatic and skeletal muscle insulin resistance notably through IL-6 since these animals increased hepatocyte expression of IL-6 and plasma IL-6 concentrations; and IL-6 neutralization (IL-6 antibody) restored insulin sensitivity in both hepatic and peripheral insulin resistance [Cai, Yuan et al. 2005]. These findings suggest that steatosis can cause sub-acute hepatocellular inflammation by activating IKKβ/NF-kB pathway, which up-regulates the production of pro-inflammatory cytokines that mediate both local hepatic and systemic insulin resistance.

The balance between various inflammatory mediators seems to play an important role in hepatic and systemic insulin action and also in the development of fatty liver disease [Tilg and Hotamisligil 2006]. For instance, it has been suggested that a proper balance

between IL-10 and TNF-α, rather than any of individual cytokines is of more physiological importance and IL-10/TNF-α ratio has been pointed out as an indicator of inflammatory status [Kaur, Sharma et al. 2006; Leonidou, Mouzaki et al. 2007]. TNF-α plays a central role in initiating and sustaining inflammation and has a pivotal role in the production of several cytokines [Ksontini, MacKay et al. 1998; Coppack 2001; Trayhurn and Wood 2005]. Whereas, IL-10 demonstrates potent anti-inflammatory properties through restraining IKKβ/NF-kB signaling pathway and inhibiting the production of various pro-inflammatory cytokines including IL-6 and TNF-α [Schottelius, Mayo et al. 1999; Bolger, Sharma et al. 2002]. Hashem *et al.* reported that IL-10/TNF-α ratio is a convenient predictive biomarker for investigation of fatty liver of different grades and suggests that modulation of this ratio in favor of increasing it may exert significant improvement [Hashem, Mahmoud et al. 2008]. However, considering the fact that IL-6 by itself is known to induce insulin resistance in hepatocytes [Kim, Higashimori et al. 2004] and according to their results on IL-6 as the strongest evidence of pathological involvement, Cai *et al.* state that ‘’many pieces of the pathogenic puzzle are still missing including how steatosis activates IKK-β and NF-κB and how IL-6 mediates hepatic insulin resistance’’ [Cai, Yuan et al. 2005].

Inflammation and menopause

The increase in visceral adiposity along with deprivation of female ovarian sex hormones across the menopausal transition may result in the development of pro-inflammatory cytokine changes, pointing to a role of cytokines and inflammation in metabolic disorders. In fact, it has been suggested that adipocytokines may be a link connecting postmenopausal hormonal changes, the excess of visceral fat and increased risk of cardiovascular disease [Sieminska, Cichon-Lenart et al. 2006]. There is now a large body of evidence suggesting that the decline in ovarian function with menopause increases pro-inflammatory cytokines such as CRP, IL-1, IL-6, and TNF-α [Pfeilschifter, Koditz et al.

2002; Vural, Akgul et al. 2006; Georgiadou and Sbarouni 2009; Kireev, Tresguerres et al. 2010]. Physiological concentrations of E2 have been shown to inhibit the spontaneous secretion of these pro-inflammatory cytokines [Rogers and Eastell 2001; Vural, Akgul et al. 2006] suggesting that estrogens have anti-inflammatory and vasoprotective properties [Miller and Duckles 2008]. However, a review paper that recently outlined the effects of hormone replacement therapy on inflammatory biomarkers states that although animal and observational studies have shown beneficial effects of hormone replacement therapy in early periods of post-menopause, randomized trials in older women have not shown any benefit in the prevention of cardiovascular events; strongly supporting the current practice which is not to prescribe HRT [Georgiadou and Sbarouni 2009].

In rodents, it has been shown that serum IL-1β increases shortly after Ovx which precedes body mass and retroperitoneal fat mass increases; indicating the fact that the interplay between ovarian function and inflammatory cytokines may not be directly related to weight and fat gain per se [Percegoni, Ferreira et al. 2009]. Moreover, inflammatory responses of different tissues in animal models of menopause have been investigated. Estrogen-deficient rats were found to have increased production of IL-6 and IL-8 in adipose tissue [Bruun, Nielsen et al. 2003]. Hamilton *et al.* found an Ovx-induced increases in myocardial genes mediating inflammation including IL-6 and TNF-α [Hamilton, Lin et al. 2008]. In the study by Kireev *et al.*, inflammation (increased pro-inflammatory cytokines TNF-α, IL-1β, IL-6 and decreased anti-inflammatory cytokine IL-10) induced during aging in the liver were more marked in castrated than in intact old female rats [Kireev, Tresguerres et al. 2010]. Administration of hormonal replacement therapy was able to inhibit the induction of pro-inflammatory cytokines in different tissues in all of these studies. More interestingly, very recently, the research group of Greenburg in Tufts University showed that increased inflammation is an early event in the development of Ovx-induced obesity and identified, for the first time, distinct depot-specific inflammatory profiles that support a role for macrophages in Ovx-associated adipose tissue inflammation; highlighting the loss of ovarian function as a metabolic risk factor that can promote a

deleterious state of chronic inflammation even in the absence of dietary change [Rogers, Perfield et al. 2009]. Ovx-induced hepatic steatosis in this study was associated with the induction of hepatic TNF-α and osteopontin (a cytokine is implicated in the progressive pathophysiology of hepatic inflammation and cancer as well as the development of insulin resistance and atherosclerosis) (Figure 7). On the other hand, it is important to recall that physical activity mediates anti-inflammatory effects in skeletal muscle and fat tissue [Bruunsgaard 2005]. However, to our knowledge, currently the effects of exercise training on Ovx-induced hepatic inflammation are entirely unknown. In humans, exercise intervention has been shown to improve the metabolic and lipid profile and to reduce inflammatory and cell adhesion molecules in postmenopausal women [Wegge, Roberts et al. 2004].

General objective of thesis and presentation of manuscripts

The general objective of this thesis is to provide information on the treatment and prevention of estrogen withdrawal-induced fat mass increase and hepatic steatosis by lifestyle modifications including diet and exercise training in Ovx rat model of menopause. Following this objective, in this chapter the emergence of metabolic syndrome (intra-abdominal fat) and hepatic steatosis in the postmenopausal hormonal state, and their treatment and prevention by lifestyle modifications such as exercise training have been reviewed. In addition, the review on the pathogenic role of sub-acute inflammation in obesity and insulin resistance was presented. In coming chapters (chapters 2-5) the experimental studies of this thesis will be presented. The first study was undertaken to evaluate the effect of resistance training protocol on body weight and fat mass in Ovx Sprague-Dawley rats following diet-induced weight loss. In line with approach, the aim of the second study was to investigate the effects of maintaining only one of the two components of a food restriction (FR) + resistance training (RT) regimen on the regain of body weight and fat mass (liver and adipocytes) in Ovx rats. The third study was conducted

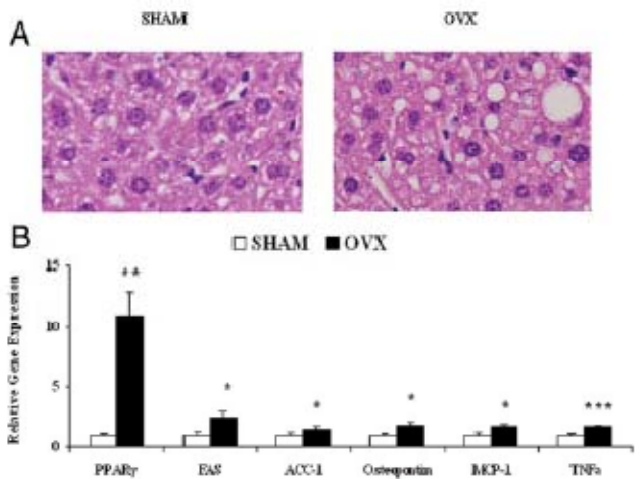

Figure 7. Taken from [Rogers, Perfield et al. 2009] Ovx mice display early hepatic steatosis and inflammation. A, Representative hematoxylin and eosin staining demonstrates more lipid droplet development in liver sections from OVX mice. B, Liver gene expression determined using quantitative real-time PCR. *Error bars* indicate SEM. n = 7–10. *, P <0.05; **, P< 0.01; ***, P< 0.001.

to determine whether a training state protects against the metabolically deleterious effects of ovariectomy on liver and adipocytes fat accumulation in rats. In continuation of this study, we hypothesized, in the fourth study that the reduction in liver fat accumulation known to occur with exercise training in Ovx rats is associated with reduced expression of genes involved in lipogenesis while favoring the expression of transcription factors regulating lipid oxidation. In this last study, we also tested the hypothesis that liver fat accumulation in Ovx rats is associated with an increased gene expression of several pro-inflammatory markers and that exercise training would attenuate this response.

Chapter 2: Original article 1

Title:

Substituting food restriction by resistance training prevents liver and body fat regain in Ovx rats.

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Substituting food restriction by resistance training prevents liver and body fat regain in Ovx rats.

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Short title: Fat regain in ovariectomized rats

Key words: Body mass, Food restriction, Exercise, Hepatic steatosis, Plasma lipids, Menopause.

Abstract

Objective: Fat mass gain and regain following weight loss is a major concern and may even be more critical after menopause. The present study was undertaken to evaluate the effect of resistance training protocol on body weight and fat mass in ovariectomized Sprague-Dawley rats following diet-induced weight loss.

Design: Rats were randomly divided into ovariectomized (Ovx) and sham-operated (Sham) groups. Five weeks after ovariectomy, Ovx rats were subjected to a 26% food restriction (OvxFR) for 8 wk. Following this period, OvxFR rats went back to a normal *ad libitum* feeding and divided into two groups: either sedentary or undergoing a resistance training program for an additional 5 weeks, which consisted of climbing a 1.5-m vertical grill, 20-40 times, with progressively increasing load 4 times/week.

Results: The food restriction program decreased ($P < 0.01$) body mass, fat pad weight (intra-abdominal and subcutaneous), and liver triacylglycerol (TAG) levels as compared to normally fed Ovx rats. Stopping the food restriction program over a 5-week period resulted in a partial regain in body weight and intra-abdominal fat pad weight ($P < 0.05$), and in an almost complete regain in liver TAG compared to normally fed Ovx rats. On the other hand, no significant increases in these variables were noted when the food restriction was replaced by resistance training over the same 5-week period.

Conclusion: These results indicate that a resistance training program could be useful in preventing body weight as well as adipose tissue and liver fat regain in Ovx rats, following diet-induced weight loss. It is suggested that alternating from a food restriction regimen to a resistance training program can be an interesting strategy to promote successful long-term weight reduction in postmenopausal women.

Introduction

Menopause is associated with increased risks of developing obesity and its associated health problems such as dyslipidemia, diabetes and cardiovascular diseases.[1] In recent years, fat accumulation in liver, often in conjunction with increased fat in intra-abdominal depots, has been identified as an important factor in the development of insulin resistance and it is now considered as the hepatic component of the metabolic syndrome.[2, 3] Recent data in ovariectomized (Ovx) rats support the interpretation that the absence of estrogens contributes to liver lipid infiltration.[4] There is also good evidence that menopause is in fact associated with the development of a state of hepatic steatosis.[5] In a recent study that included 800 women aged 40-59 years, it was confirmed that the menopausal status is indeed associated with a state of hepatic steatosis.[6] Therefore, information regarding fat accumulation in liver and adipocytes is needed to establish strategies to counteract the deleterious metabolic effects of increased body fat content in postmenopausal women.

Weight loss achieved through lifestyle modifications (diet and exercise) may reduce the risks of developing obesity-induced disorders in post-menopausal women.[7, 8] It has been reported that weight loss, as modest as 5 to 10%, can reduce obesity-related disorders.[9] However, long-lasting maintenance of weight loss is difficult to achieve and body weight relapse is a common feature after weight loss intervention.[10-12] Strategies to improve the maintenance of weight loss and its associated metabolic changes are multiple but most of them involve changes in diet and higher levels of physical activity.[13-15] Since weight loss achieved through restrictive diet has negative effects on muscle mass,[16] some authors used a resistance training program in conjunction with energy restriction in postmenopausal women with [17] and without [18] beneficial effects on body weight and fat mass. In a recent study, an Ovx rat model was used to evaluate the effects of adding resistance training to a restrictive diet. In this study, it was found that the addition of resistance training to food restriction not only further reduced fat accumulation in the intra-abdominal depots, but completely abolished liver lipid accumulation in the Ovx rats.[19] It is thus possible that resistance training may constitute an interesting adjunct therapy to food restriction to

maintain the beneficial effects of food restriction in estrogen-deficient animals and in postmenopausal women. In the present study, we used an Ovx rat model to test 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.

Methods

Animal care. Six-week-old Sprague-Dawley female rats, ranging in mass from 180 to 200 g were purchased from Charles River (St-Constant, PQ, Canada) and maintained on a 12:12 h light-dark cycle, with lights on at 0600. The animals were housed individually throughout the study at 20-23°C ambient temperature. All rats received standard rat chow (12.5% fat; 63.2% carbohydrate; 24.3% protein; kJ, Agribrands Purina Canada, Woodstock, ON, Canada) and had free access to tap water. Rats were randomly divided into seven groups (n = 8 per group) that initially had similar mean body masses and were treated similarly in terms of daily manipulations. All experiments described in this report were conducted according to the guidelines of the Canadian Council on Animal Care after institutional approval.

Surgery. Two days after their arrival to our laboratory, rats were randomly divided into ovariectomized (Ovx) and sham-operated (Sham) groups. Ovx was conducted according to the technique described by Robertson and colleagues.[20] Animals were injected with antibiotics (Tribrissen 24%; 0.125cc/kg s.c.) for 3 days, beginning the day before surgery. We followed the surgery guidelines that have been described in the report of Shinoda and colleagues.[21] Following surgery, body weight and food intake were monitored three times per week in all rats.

Groups, food restriction and training protocol. A schematic representation of the experimental design is presented in Figure 1. Five weeks after ovariectomy, rats were first submitted to a food restriction regimen (OvxFR) for 8 weeks (weeks 6-13). Following this period, OvxFR rats went back to a normal *ad libitum* feeding while either remaining sedentary (OvxPostFR) or being submitted to a resistance training program (OvxPostFR+RT) for an additional 5 weeks (weeks 14-18). Subgroups of Sham and Ovx rats were sacrificed either after 13 or 18 weeks. The food restriction program consisted of a target 25% daily caloric reduction calculated from the daily energy intake average of the 2 preceding weeks in normally fed rats. It turned out that the daily caloric reduction was 26% for the rats submitted to this program for 8 weeks (weeks 6-13). Resistance training was

conducted using a device similar to the one described by Murphy and colleagues.[22] The resistance training program consisted of climbing on a 1.5-m vertical metal grill with a slope of 7°, four times a week with a progressively increasing load up to 50% of body weight attached to the tail. The number of repetitions increased progressively during the first 2 weeks from two sets of ten repetitions to four sets of ten repetitions. Each repetition lasted ~3-5 s with ~40 s rest between repetitions. Exercise animals were sacrificed 48 h after the last exercise session.

Sacrifice. Rats were sacrificed between 09.00 and 12.00. Remaining food was removed from the animal's cage at least 3 h before sacrifice. Immediately after complete anesthesia (pentobarbital sodium; 50mg/kg i.p.), the abdominal cavity was opened following the median line of the abdomen, and approximately 4 ml of blood were collected from the abdominal vena cava (<45s) into syringes pre-treated with ethylenediaminetetraacetic acid (15%, EDTA). Blood was centrifuged at 3000 rpm at 4°C for 12 min (Beckman GPR Centrifuge) and the plasma was kept for further analysis. Several organs and tissues were removed and weighed (Mettler AE 100) in the following order: liver, uterus, mesenteric, urogenital, retroperitoneal and subcutaneous fat deposits along with four skeletal muscles of the right hind-limb (soleus, plantaris, medial gastrocnemius and lateral gastrocnemius). All tissue samples were frozen in liquid nitrogen immediately after they were weighed. The liver median lobe was freeze-clamped and used for triacylglycerol (TAG) determination. Mesenteric fat was collected from the superficial area covering the alimentary tract, the spleen and the pancreas. Special care was taken to distinguish fat cells from pancreatic cells, based on color and texture differences. On the right side of the animal, the subcutaneous fat was removed from the region between the caudal border of the rib cage, the dorsal and ventral midlines of the body and the urogenital organs. All tissue and plasma samples were stored at -78°C until analyses were performed. All rats were visually inspected for presence or not of ovaries, and uteri were excised and weighed to confirm ovariectomy or sham surgery.

Analytical procedures. Plasma 17β-estradiol and insulin concentrations were determined with radioimmunoassay test kits distributed by ICN Biomedicals (Costa Mesa, CA, USA) and Medicorp Laboratories (Montreal, PQ, Canada), respectively. Plasma glucose concentrations were determined with the use of a glucose analyzer (Yellow Springs Instruments 2300, Yellow Springs, OH, USA). Plasma glucose and insulin values were used to calculate a homeostasis model assessment of insulin resistance (HOMA-IR) as followed: glucose (mmol/l) x insulin (mIU/ml)/22.5.[23] Plasma free fatty acid (FFA) and TAG concentrations were determined with an enzymatic colorimetric assay available from Roche Diagnostics (Mannheim, Germany) and Sigma (Saint Louis, Missouri, USA), respectively. Liver TAG concentrations were estimated from glycerol released after ethanolic KOH hydrolysis by using commercial kit from Sigma (Saint Louis, Missouri, USA).

Statistical analysis. Values are expressed as means ± standard error (SE). Statistical analyses were performed by one-way ANOVA for non-repeated measures design using treatment as a main effect. These analyses were conducted separately at two different times (13 and 18 weeks). Fisher's post hoc test was used in the event of a significant ($P < 0.05$) F ratio. The effect of ovariectomy on food intake between weeks 1 and 5 was analyzed using unpaired t-test.

Results

Final body weight and food intake. Ovariectomized as compared to Sham animals had higher body weight, measured either after 13 and 18 weeks (Figure 2a). Food restriction between weeks 5 and 13 led to significantly ($P < 0.01$) lower final body weight in Ovx rats than that observed in the free-feeding Ovx animals. In fact, body weight in OvxFR animals was not significantly different from that measured in Sham rats at 13 weeks. Interrupting the food restriction treatment between weeks 13 and 18 resulted in body weight values that were still lower ($P < 0.05$) than values measured in free-feeding Ovx rats but higher ($P < 0.01$) than Sham rats (Figure 1a). This indicates a partial regain of body weight with the abandonment of the food restriction program. However, the final body weight in Ovx rats in which food restriction was substituted by resistance training between weeks 13 and 18 was not significantly different that of Sham rats but significantly lower than that observed in both OvxPostFR and Ovx ($P < 0.01$).

As for body weight, food intake was higher ($P < 0.01$) in Ovx than in Sham rats during the first 5 weeks after surgery (Figure 2b). As designed, the 8-week food restriction program resulted in a lower ($P < 0.01$) food intake in OvxFR compared to *ad libitum* fed Ovx rats. However, all groups of animals had similar food intake from weeks 14 to 18.

Uterus weight, plasma estradiol and muscle weight. All Ovx animals showed lower ($P < 0.01$) uterus weight and plasma estradiol concentrations than Sham rats, indicating total ovariectomy (Table 1). Ovx resulted in higher ($P < 0.05$) muscle weight compared to Sham animals and these higher values were maintained whether Ovx rats underwent the food restriction or the resistance training treatment. This relation was, however, abolished when the sum of muscle weight was divided by body weight. The gain in muscle weight with ovariectomy was observed only in conditions where body weight in Ovx rats was reduced, that is, after food restriction and when food restriction was substituted by resistance training.

Plasma glucose, insulin and free fatty acids concentrations. There was no difference in plasma glucose concentration after 13 weeks (Table 2). After 18 weeks, however, plasma glucose concentrations were higher ($P < 0.01$) in Ovx and OvxPostFR rats compared to Sham animals; this result was attenuated by substituting food restriction by resistance training ($P < 0.05$). The food restriction regimen and substitution of food restriction by resistance training in OvxFR and OvxPostFR+RT rats, respectively, led to lower plasmatic concentrations of insulin compared to Ovx rats. Calculation of the HOMA-IR ratio indicated lower values in OvxFR compared to Ovx rats at week 13. Interestingly, the resistance training program in OvxPostFR+RT animals was associated with lower HOMA-IR ratio compared to Ovx and OvxPostFR rats. There were no inter-group differences in plasma free fatty acid levels (Table 2).

Liver and plasma TAG concentrations. Ovarictomy resulted in significantly ($P < 0.01$) higher levels of liver TAG measured either after 13 and 18 weeks (Figure 3a). Food restriction between weeks 5 and 13 significantly ($P < 0.01$) attenuated liver lipid infiltration in Ovx rats such that no significant differences were found compared to Sham rats. Abandoning the food restriction regimen between weeks 13 and 18 resulted in a regain in liver fat levels that were midway between those measured in Sham and Ovx animals. However, substituting food restriction by resistance training kept liver TAG levels in OvxPostFR+RT rats to the level of Sham rats. Similar findings were observed for plasma TAG levels (Figure 3b). Plasma TAG levels were not significantly different between Ovx and Sham rats after 13 weeks but were lower in Ovx than in Sham animals after 18 weeks. The FR regimen significantly ($P < 0.01$) reduced plasma TAG accumulation in Ovx rats (week 13). Plasma TAG levels returned to the level of normally fed Ovx rats 5 weeks after abandoning the food restriction treatment. Interestingly, however, substituting food restriction by resistance training prevented the re-increase in plasma TAG levels.

Intra-abdominal and subcutaneous fat pad weights. The sum of three intra-abdominal fat pad weight and subcutaneous fat depots were higher in Ovx than in Sham rats either after 13 ($P < 0.01$) or 18 weeks ($P < 0.05$) (Figure 4). Similarly to what we

observed for liver TAG levels, food restriction significantly ($P < 0.01$) reduced fat depots in adipose tissues in Ovx rats. Interrupting of the food restriction regimen during 5 weeks did not result in a pronounced regain of fat mass such that fat pad weights were still lower ($P < 0.05$) than what was measured in Ovx rats. However, substituting food restriction by resistance training largely lowered ($P < 0.01$) fat mass accumulation.

Discussion

In this study, we sought to determine the efficacy of resistance training in attenuating the regain of adiposity and liver fat after the cessation of a prolonged food restriction leading to weight loss in Ovx rats. Results of this experimental approach indicate that substitution of resistance training favored the maintenance of a lower body weight and adiposity in this animal model. These data support the concept that resistance training may provide a successful alternative to chronic food restriction to avoid relapse of body weight and lipid accumulation in Ovx rats.

Body weight. As previously reported, body weight was higher in Ovx than in Sham rats.[24, 25] The ~ 25% FR in OvxFR animals resulted in a reduction of body weight to the level of Sham animals (Figure 2a, week 13). Considering that food intake in OvxFR animals was lower than in Sham rats, one would have expected a larger reduction in body weight. This suggests a decrease in energy expenditure in the OvxFR rats. As expected, the abandonment of the food restriction regimen resulted in a regain of body weight, but not completely since body weight in OvxPostFR rats was still lower than in Ovx animals. Nevertheless, this partial regain in body weight was significantly attenuated by substituting resistance training for food restriction. These changes in body weight can hardly be attributed to differences in food intake since food intake was similar in all groups after the abandonment of the food restriction program (Figure 2b, weeks 14-18). It is well documented that weight-reduced rats show a profound drive to regain lost weight when they are allowed free access to food.[26, 27] A large body of evidence now suggests that a homeostatic feedback system defending body weight and adiposity is fundamental to this metabolic drive to regain lost weight.[28-30] Interestingly, it has also been reported that exercise lowers the defended level of weight gain and adiposity.[31] These authors suggested that exercise resets the homeostatic balance between energy intake and expenditure toward defense of a lower level of weight gain and adiposity. It seems that exercise produces a different set of regulatory signals from caloric restriction that lowers the defended level of weight gain and adiposity.[31] In a recent study,[32] the same authors showed that early-onset

exercise, as opposed to early caloric restriction, may favorably alter the development of the hypothalamic pathways controlling energy homeostasis during brain development. It may, therefore, be inferred that the introduction of the resistance training program initiated a new set of regulatory signals that contributes to the maintenance and even the lowering of the defended body weight gain and adiposity after cessation of the food restriction regimen.

Liver TAG and adiposity. The main objective of the present study was to determine whether substituting food restriction by resistance training would help to maintain the decreased liver lipid infiltration that is likely to occur with food restriction in Ovx rats. The importance of this information has been highlighted in recent years by the recognition of hepatic steatosis as the hepatic component of the metabolic syndrome.[33] Ovx in the present study resulted in ~ 50-55% higher fat accumulation in the livers of our rats (Figure 3a). Food restriction resulted in a complete resorption of liver fat accumulation to the level of that in Sham rats (week 13), along with an important reduction in plasma TAG. In a recent study,[19] only a partial reduction of liver TAG levels was found after 8 weeks of food restriction in Ovx rats. The discrepancy between the two studies may be due to a better control of the food restriction regimen in the present study. The abandonment of the food restriction resulted in regains of liver and plasma TAG that were significantly attenuated by the introduction of the resistance training program. It was previously reported that the adjunct of a resistance training program to a food restriction regimen largely contributed to the reduction of fat accumulation in liver of Ovx rats.[19] The present data complement this finding by indicating that a food restriction regimen may be adequately replaced by a resistance training program to maintain low liver fat accumulation in Ovx animals. Although the present study did not address mechanisms involved in these responses, it is likely that the mechanisms of action are different for the food restriction than for the resistance training treatment. Food intake reduction in the present Ovx rats was important, reaching food intake levels lower than what was ingested in Sham rats. This must have substantially reduced the amount of substrate taking up by the liver and consequently resulted in a progressive resorption of accumulated lipids. A return to a normal diet would increase the amount of substrate available, thus causing a regain of liver fat accumulation.

The introduction of resistance training after cessation of food restriction would, on the other hand, control liver fat accumulation by stimulating lipid oxidation inside the liver itself, in addition of increasing energy expenditure. This interpretation is supported by the observation that the introduction of the resistance training program after cessation of the food restriction regimen not only prevented the relapse in fat accumulation in adipose tissues, but reduced fat mass to levels lower than those measured in Ovx animals. Since changes in liver fat accumulation paralleled changes in adipose tissue fat mass following food restriction and resistance training in Ovx rats, one may reach the conclusion that liver fat accumulation is strictly related to adipose tissue fat accumulation or relapse. Although this is probably correct to a certain point, it must be kept in mind that estrogens can act intrahepatically as a protective tool against liver lipid infiltration. Estrogens are known to have a role in regard to several regulatory aspects of hepatic lipid metabolism, including very low density lipoprotein synthesis.[34, 35] The absence of estrogens as such could therefore favor liver fat accumulation. If, on one hand, food restriction would most likely reduce the amount of lipids taken up by the liver, on the other hand, it is possible that resistance training may stimulate metabolic pathways in liver common to estrogens, thus favoring a reduction in hepatic fat accumulation. In this regard, endurance training in Ovx rats has been recently reported to have beneficial effects similar to those of estrogens on the heart of Ovx rats.[36]

Food restriction in Ovx rats was associated with lower plasma insulin levels compared to *ad libitum* fed Ovx rats. However, this effect was not observed anymore after cessation of food restriction, indicating an increase in insulin levels. Plasma glucose levels were also higher with cessation of food restriction as compared to values in Sham rats. Most interestingly, it is with the introduction of the resistance training program that the lower plasma glucose and insulin values were observed. Although our rats were not in an overnight fasted state (3-h fast or more), we calculated HOMA-IR to obtain an index of insulin sensitivity. This index indicates a deterioration of insulin sensitivity with Ovx (week 13), which was improved by the food restriction regimen and even more by the resistance training program. Although insulin sensitivity in the present context should be measured

using more sophisticated techniques, the present data suggest that, in addition to attenuating fat accumulation resulting from cessation of a food restriction regimen, the introduction of a resistance training program in Ovx animals may also be an adjunct in attenuating the metabolic deleterious effects associated with the withdrawal of ovarian hormones.

The appropriateness of present resistance training program in Ovx animals needs to be addressed. It is difficult to use a RT program in rats without mobilizing several muscle groups. Taken into account the very short active time (~ 3-5 s) interspersed by a relatively long period of rest (~ 40 s), we feel confident that the nature of the present training program is closely related to resistance training. In the present resistance training program, rats were trained four times a week with a maximal load attached to the tail of the rats of 50% body weight, compared to five times a week with a maximal load of 75% body weight in a previous study.[19] It was thought that these limitations make the program more suitable to Ovx rats. Both of these resistance training programs did not result in higher muscle mass compared to Ovx. This may be explained by the fact that ovariectomy itself resulted in an increased muscle mass, attributed to increased body weight.[25] Accordingly, there was no difference in muscle mass/body weight between Sham and Ovx rats. The abandonment of the food restriction regimen resulted in a tendency ($P < 0.09$) to lower relative muscle mass values (0.73 vs 0.65). However, substituting food restriction by resistance training resulted in the maintenance of the relative muscle mass (0.73 vs 0.71). This may be taken as an indication that the present resistance training program had an effect on muscle mass.

In summary, results of the present study show that the cessation of food restriction regimen aimed at lower body weight and fat accumulation in Ovx rats may be advantageously substituted by a resistance training program, without causing any appreciable regain of body weight and fat in liver and adipose tissue. The resistance training program was even associated with a lowering of fat accumulation in adipocytes to levels lower than measured in Sham rats.

While it is still unclear whether the menopause transition itself brings about weight gain,[37] there is good evidence that menopause is associated with weight gain and changes in

fat distribution that increase the risk of cardiovascular diseases.[38, 39] There is also recent evidence that withdrawal of estrogens results in rapid fat accumulation in liver of postmenopausal women.[6] Postmenopausal women are, therefore, often confront with the undertaking of lifestyle modifications (nutrition and exercise) that are so hard to maintain in practice. Results of the present study suggest that changing from a food restriction regimen to a resistance training program may prove to be an interesting strategy to promote successful long-term weight reduction in postmenopausal women.

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Conflict of interest

None.

References

1. Carr MC. The emergence of the metabolic syndrome with menopause. *J Clin Endocrinol Metab* 2003;88:2404-2411.

2. Samuel VT, Liu ZX, Qu X, et al. Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. *J Biol Chem* 2004;279:32345-32353.

3. Marchesini G, Marzocchi R, Agostini F, Bugianesi E. Nonalcoholic fatty liver disease and the metabolic syndrome. *Curr Opin Lipidol* 2005;16:421-427.

4. Paquette A, Shinoda M, Rabasa Lhoret R, Prud'homme D, Lavoie J-M. Time course of liver lipid infiltration in ovariectomized rats: Impact of a high-fat diet. *Maturitas* 2007;58:182-190.

5. Clark JM. Weight loss as a treatment for nonalcoholic fatty liver disease. *J Clin Gastroenterol* 2006;40:S39-43.

6. Volzke H, Schwarz S, Baumeister SE, et al. Menopausal status and hepatic steatosis in a general female population. *Gut* 2007;56:594-595.

7. Park HS, Lee KU. Postmenopausal women lose less visceral adipose tissue during a weight reduction program. *Menopause* 2003;10:222-227.

8. Turcato E, Zamboni M, De Pergola G, et al. Interrelationships between weight loss, body fat distribution and sex hormones in pre- and postmenopausal obese women. *J Intern Med* 1997;241:363-372.

9. Ditschuneit HH, Flechtner-Mors M. Value of structured meals for weight management: Risk factors and long-term weight maintenance. *Obes Res* 2001;9 Suppl 4:284S-289S.

10. Westerterp-Plantenga MS, Kempen KP, Saris WH. Determinants of weight maintenance in women after diet-induced weight reduction. *Int J Obes Relat Metab Disord* 1998;22:1-6.

11. Pasman WJ, Saris WH, Westerterp-Plantenga MS. Predictors of weight maintenance. *Obes Res* 1999;7:43-50.

12. Glenny AM, O'Meara S, Melville A, Sheldon TA, Wilson C. The treatment and prevention of obesity: A systematic review of the literature. *Int J Obes Relat Metab Disord* 1997;21:715-737.

13. Leermakers EA, Perri MG, Shigaki CL, Fuller PR. Effects of exercise-focused versus weight-focused maintenance programs on the management of obesity. *Addict Behav* 1999;24:219-227.

14. Svendsen OL, Hassager C, Christiansen C. Six months' follow-up on exercise added to a short-term diet in overweight postmenopausal women--effects on body composition, resting metabolic rate, cardiovascular risk factors and bone. *Int J Obes Relat Metab Disord* 1994;18:692-698.

15. Andersen RE, Wadden TA, Bartlett SJ, Zemel B, Verde TJ, Franckowiak SC. Effects of lifestyle activity vs structured aerobic exercise in obese women: A randomized trial. *JAMA* 1999;281:335-340.

16. Villareal DT, Apovian CM, Kushner RF, Klein S. Obesity in older adults: Technical review and position statement of the american society for nutrition and naaso, the obesity society. *Obes Res* 2005;13:1849-1863.

17. Ryan AS, Pratley RE, Elahi D, Goldberg AP. Changes in plasma leptin and insulin action with resistive training in postmenopausal women. *Int J Obes Relat Metab Disord* 2000;24:27-32.

18. Joseph LJ, Trappe TA, Farrell PA, et al. Short-term moderate weight loss and resistance training do not affect insulin-stimulated glucose disposal in postmenopausal women. *Diabetes Care* 2001;24:1863-1869.

19. Corriveau P, Paquette A, Brochu M, Prud'homme D, Rabasa-Lhoret R, Lavoie J-M. Resistance training prevents liver fat accumulation in ovariectomized rats. *Maturitas* 2008;59:259-267.

20. Robertson MC, Owens RE, Klindt J, Friesen HG. Ovariectomy leads to a rapid increase in rat placental lactogen secretion. *Endocrinology* 1984;114:1805-1811.

21. Shinoda M, Latour MG, Lavoie J-M. Effects of physical training on body composition and organ weights in ovariectomized and hyperestrogenic rats. *Int J Obes Relat Metab Disord* 2002;26:335-343.

22. Murphy RJ, Beliveau L, Seburn KL, Gardiner PF. Clenbuterol has a greater influence on untrained than on previously trained skeletal muscle in rats. *Eur J Appl Physiol Occup Physiol* 1996;73:304-310.

23. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412-419.

24. McElroy JF, Wade GN. Short- and long-term effects of ovariectomy on food intake, body weight, carcass composition, and brown adipose tissue in rats. *Physiol Behav* 1987;39:361-365.

25. Richard D. Effects of ovarian hormones on energy balance and brown adipose tissue thermogenesis. *Am J Physiol* 1986;250:R245-249.

26. Hill JO, Thacker S, Newby D, Sykes MN, Digirolamo M. Influence of food restriction coupled with weight cycling on carcass energy restoration during ad-libitum refeeding. *Int J Obes* 1988;12:547-555.

27. Corbett SW, Wilterdink EJ, Keesey RE. Resting oxygen consumption in over- and underfed rats with lateral hypothalamic lesions. *Physiol Behav* 1985;35:971-977.

28. Levin BE, Dunn-Meynell AA. Defense of body weight against chronic caloric restriction in obesity-prone and -resistant rats. *Am J Physiol Regul Integr Comp Physiol* 2000;278:R231-237.

29. MacLean PS, Higgins JA, Johnson GC, et al. Enhanced metabolic efficiency contributes to weight regain after weight loss in obesity-prone rats. *Am J Physiol Regul Integr Comp Physiol* 2004;287:R1306-1315.

30. MacLean PS, Higgins JA, Johnson GC, Fleming-Elder BK, Peters JC, Hill JO. Metabolic adjustments with the development, treatment, and recurrence of obesity in obesity-prone rats. *Am J Physiol Regul Integr Comp Physiol* 2004;287:R288-297.

31. Levin BE, Dunn-Meynell AA. Chronic exercise lowers the defended body weight gain and adiposity in diet-induced obese rats. *Am J Physiol Regul Integr Comp Physiol* 2004;286:R771-778.

32. Patterson CM, Dunn-Meynell AA, Levin BE. Three weeks of early-onset exercise prolongs obesity resistance in dio rats after exercise cessation. *Am J Physiol Regul Integr Comp Physiol* 2008;294:R290-301.

33. Kotronen A, Westerbacka J, Bergholm R, Pietilainen KH, Yki-Jarvinen H. Liver fat in the metabolic syndrome. *J Clin Endocrinol Metab* 2007;92:3490-3497.

34. D'Eon TM, Souza SC, Aronovitz M, Obin MS, Fried SK, Greenberg AS. Estrogen regulation of adiposity and fuel partitioning. Evidence of genomic and non-genomic regulation of lipogenic and oxidative pathways. *J Biol Chem* 2005;280:35983-35991.

35. Srivastava RA, Tang J, Baumann D, Schonfeld G. Hormonal and nutritional stimuli modulate apolipoprotein b mrna editing in mouse liver. *Biochem Biophys Res Commun* 1992;188:135-141.

36. Gutkowska J, Paquette A, Wang D, Lavoie J-M, Jankowski M. Effect of exercise training on cardiac oxytocin and natriuretic peptide systems in ovariectomized rats. *Am J Physiol Regul Integr Comp Physiol* 2007;293:R267-275.

37. Dubnov-Raz G, Pines A, Berry EM. Diet and lifestyle managing postmenopausal obesity. *Climacteric* 2007;10:38-41.

38. Astrup A. Physical activity and weight gain and fat distribution changes with menopause: current evidence and research issues. *Med Sc Sports Exerc* 1999;31:S564-S567.

39. Genazzani AR, Gambacciani M. Effect of climacteric transition and hormone replacement therapy on body weight and body fat distribution. *Gynecol Endocrinol* 2006;22:145-150.

Table 1. Uterus weight, estradiol levels, and sum of weight of four muscles measured after 13 and 18 weeks in the following groups of rats: Sham (sham-operated), Ovx (ovariectomized), OvxFR (5-week ovariectomized + 8-week food restriction), OvxPostFR (5-week ovariectomized + 8-week food restriction + 5-week interruption of food restriction), and OvxPostFR+RT (5-week ovariectomized + 8-week food restriction + 5-week resistance training). Values are given as mean ± standard error with $n = 8$ rats in each group

	Sham	*Ovx*	*OvxFR*	*OvxPostFR*	*OvxPostFR + RT*
Uterus weight (g)					
Week 13	$0.68 \pm 0.06^{b,e}$	0.104 ± 0.01	0.112 ± 0.01		
Week 18	$0.64 \pm 0.06^{b,g}$	0.102 ± 0.01		0.093 ± 0.01	0.098 ± 0.01^{d}
Estradiol (pg/ml)					
Week 13	$157 \pm 23^{b,e}$	72 ± 4	68 ± 4		
Week 18	$121 \pm 17^{b,g}$	67 ± 4		63 ± 3	70 ± 10^{d}
Sum of muscle weight (g)					
Week 13	$2.08 \pm 0.06^{b,e}$	2.46 ± 0.07	2.42 ± 0.08		
Week 18	$2.10 \pm 0.04^{b,f}$	2.49 ± 0.11		2.46 ± 0.13	2.42 ± 0.09^{c}
Sum of muscle weight/body weight					
Week 13	0.67 ± 0.02	0.65 ± 0.02	$0.73 \pm 0.02^{b,c}$		
Week 18	0.63 ± 0.02	0.61 ± 0.02		0.65 ± 0.03	$0.71 \pm 0.02^{b,c}$

Significantly different from Ovx group, $^{b}P < 0.01$; significantly different from Sham group, $^{c}P < 0.05$, $^{d}P < 0.01$; significantly different from OvxFR group, $^{e}P < 0.01$; significantly different from OvxPostFR group, $^{f}P < 0.01$, $^{g}P < 0.01$

Table 2. Plasma concentrations of glucose, insulin, HOMA-IR, and free fatty acids measured after 13 and 18 weeks in the following groups of rats: Sham (sham-operated), Ovx (ovariectomized), OvxFR (5-week ovariectomized + 8-week food restriction), OvxPostFR (5-week ovariectomized + 8-week food restriction + 5-week interruption of food restriction), and OvxPostFR+RT (5-week ovariectomized + 8-week food restriction + 5-week resistance training). Values are given as mean ± standard error with n = 8 rats in each group

	Sham	*Ovx*	*OvxFR*	*OvxPostFR*	*OvxPostFR + RT*
Glucose (mmol/l)					
Week 13	8.6 ± 0.4	9.3 ± 0.3	9.4 ± 0.5		
Week 18	8.0 ± 0.1[b]	9.8 ± 0.4		9.5 ± 0.2[d]	8.7 ± 0.3[a]
Insulin (pmol/l)					
Week 13	403 ± 42	532 ± 53	345 ± 44[a]		
Week 18	398 ± 49	407 ± 35		390 ± 27	270 ± 44[c,a,f]
HOMA-IR					
Week 13	25.8 ± 2.9[a]	37.2 ± 3.5	24.1 ± 3.4[a]		
Week 18	23.8 ± 3	30.7 ± 3.3		27.5 ± 2.2	17.7 ± 3.1[b,f]
Free fatty acids (mmol/l)					
Week 13	0.093 ± 0.01	0.073 ± 0.01	0.086 ± 0.02		
Week 18	0.125 ± 0.02	0.101 ± 0.02		0.091 ± 0.02	0.056 ± 0.01

Significantly different from Ovx group, [a]$P < 0.05$, [b]$P < 0.01$; significantly different from Sham group, [c]$P < 0.05$, [d]$P < 0.01$; significantly different from OvxPostFR group, [f]$P < 0.05$

HOMA-IR, homeostatic model assessment of insulin resistance (glucose (mmol/l) × insulin (mIU/ml)/22.5; 3-h fasted state)

Legends

Figure 1. Schematic representation of the experimental design. The tip of the arrow indicates the time of sacrifice. Sham, sham-operated; Ovx, ovariectomized; FR, food restriction; RT, resistance training

Figure 2. Body weight (a) and average daily food intake (b) measured after 5, 13 and 18 weeks in the following groups of rats: Sham (sham-operated), Ovx (ovariectomized), OvxFR (5-week ovariectomized + 8-week food restriction), OvxPostFR (5-week ovariectomized + 8-week food restriction + 5-week interruption of food restriction), and OvxPostFR+RT (5-week ovariectomized + 8-week food restriction + 5-week resistance training). Values are given as mean ± standard error with $n = 8$ rats in each group. Significantly different from Ovx group, $^{a}P < 0.05$, $^{b}P < 0.01$; significantly different from OvxFR group, $^{e}P < 0.01$; significantly different from OvxPostFR group, $^{g}P < 0.01$

Figure 3. Liver triacylglycerol (TAG) concentrations (a) and plasma concentrations of TAG (b) measured after 13 and 18 weeks in the following groups of rats: Sham (sham-operated), Ovx (ovariectomized), OvxFR (5-week ovariectomized + 8-week food restriction), OvxPostFR (5-week ovariectomized + 8-week food restriction + 5-week interruption of food restriction), and OvxPostFR+RT (5-week ovariectomized + 8-week food restriction + 5-week resistance training). Values are given as mean ± standard error with $n = 8$ rats in each group. Significantly different from Ovx group, $^{a}P < 0.05$, $^{b}P < 0.01$; significantly different from Sham group, $^{d}P < 0.01$; significantly different from OvxFR group, $^{e}P < 0.01$; significantly different from OvxPostFR group, $^{f}P < 0.05$, $^{g}P < 0.01$

Figure 4. Sum of weights of three intra-abdominal (mesenteric, urogenital and retroperitoneal) fat depots (a) and subcutaneous fat depot weight (b) measured after 13 and

18 weeks in the following groups of rats: Sham (sham-operated), Ovx (ovariectomized), OvxFR (5-week ovariectomized + 8-week food restriction), OvxPostFR (5-week ovariectomized + 8-week food restriction + 5-week interruption of food restriction), and OvxPostFR+RT (5-week ovariectomized + 8-week food restriction + 5-week resistance training). Values are given as mean ± standard error with n = 8 rats in each group. Significantly different from Ovx group, $^{a}P < 0.05$, $^{b}P < 0.01$; significantly different from Sham group, $^{d}P < 0.01$; significantly different from OvxPostFR group, $^{g}P < 0.01$

Figure 1.

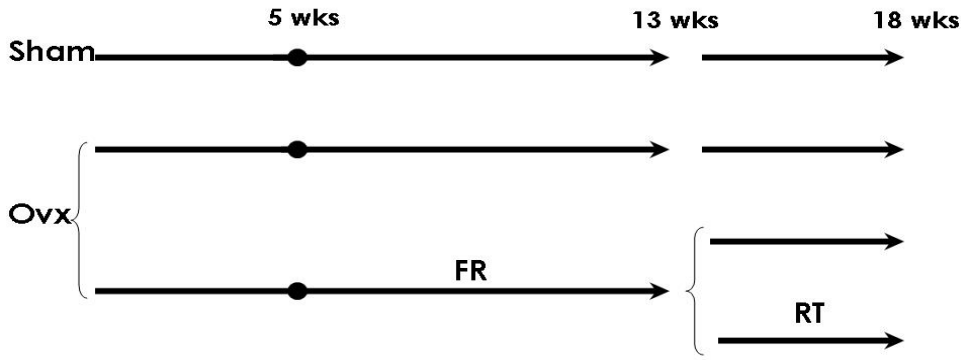


Figure 2.

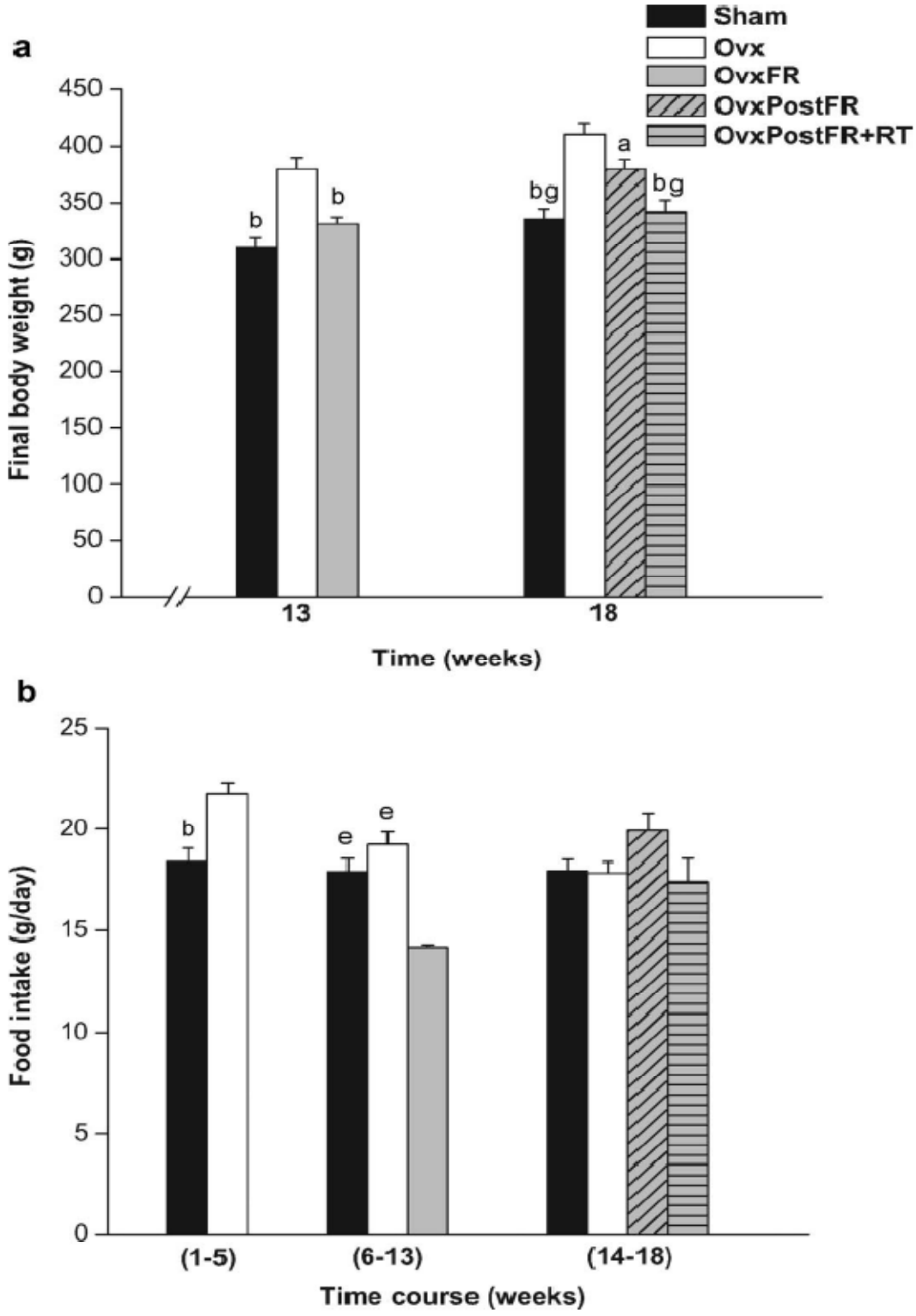

a
Sham
Ovx
OvxFR
OvxPostFR
OvxPostFR+RT
Final body weight (g)
450
400
350
300
250
200
150
100
50
0
b
b
bg
a
bg
13
18
Time (weeks)
b
Food intake (g/day)
25
20
15
10
5
0
b
e
e
(1-5)
(6-13)
(14-18)
Time course (weeks)

Figure 3.

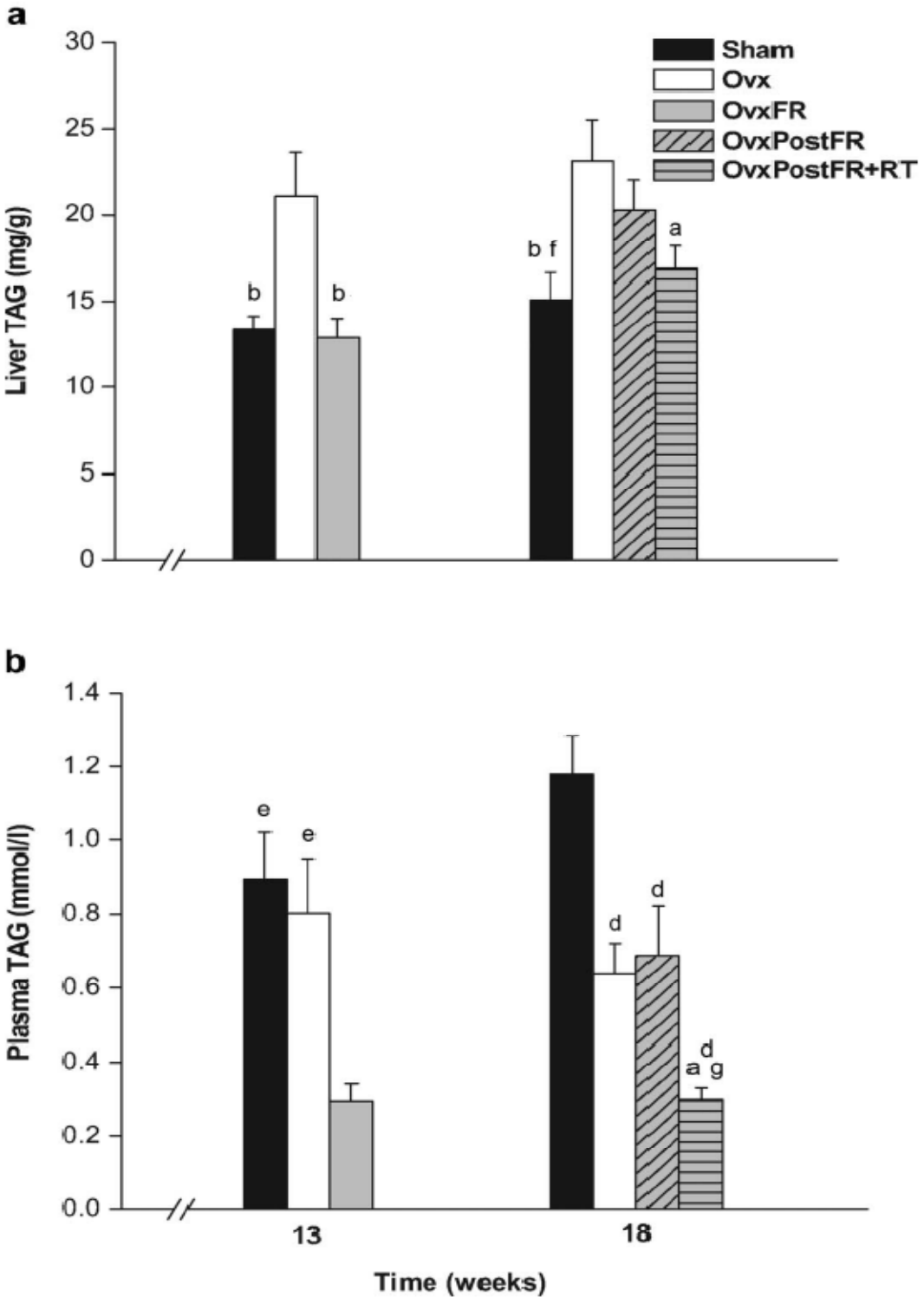
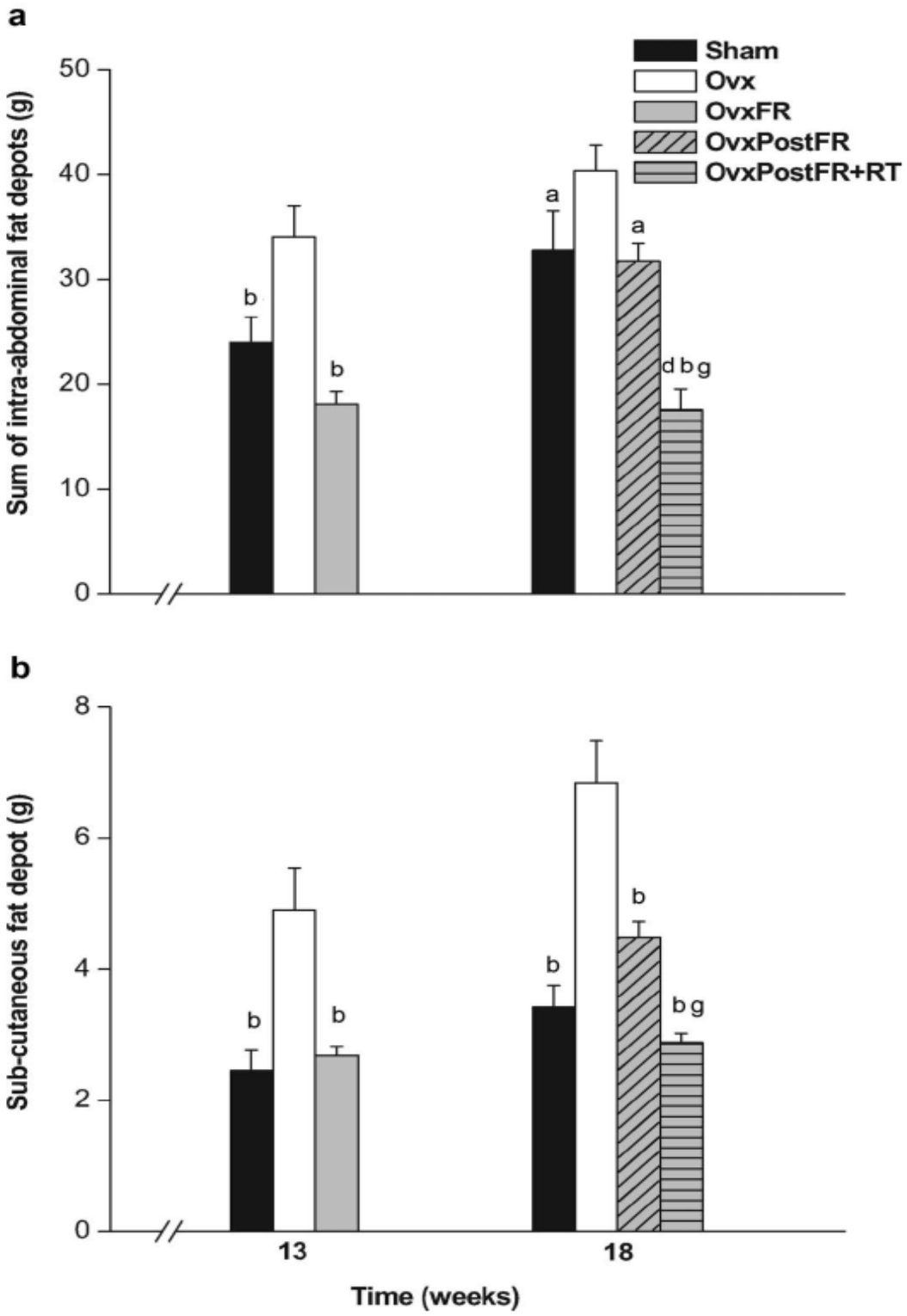

a
Liver TAG (mg/g)
30
25
20
15
10
5
0
Sham
Ovx
OvxFR
OvxPostFR
OvxPostFR+RT
b
b
b f
a
b
Plasma TAG (mmol/l)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
e
e
d
d
a g
13
18
Time (weeks)

Figure 4.


a
Sham
Ovx
OvxFR
OvxPostFR
OvxPostFR+RT
Sum of intra-abdominal fat depots (g)
50
40
30
20
10
0
b
b
a
a
d b g
b
Sub-cutaneous fat depot (g)
8
6
4
2
0
b
b
b
b
b g
13
18
Time (weeks)

Chapter 3: Original article 2

Title:

Resistance training attenuates fat mass regain after weight loss in ovariectomized rats.

Authors:

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Resistance training attenuates fat mass regain after weight loss in ovariectomized rats.

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Running title: Body weight and fat regain in ovariectomized rats

Abstract

Objective: The aim of the present study was to investigate the effects of maintaining only one of the two components of a food restriction (FR) + resistance training (RT) regimen on the regain of body weight and fat mass (liver and adipocytes) in ovariectomized (Ovx) rats.

Methods: Five week Ovx rats were submitted to a weight loss program consisting of a 26% FR combined with RT (OvxFR+RT) for 8 weeks. RT consisted of climbing a 1.5 m vertical grid with a load attached to the tail, 20-40 times with progressively increasing loads 4 times/week. Following this weight loss intervention, OvxFR+RT rats were sub-divided into 3 groups for an additional 5 weeks: 2 groups went back to a normal *ad libitum* feeding with or without RT and the other group kept only FR.

Results: Combined FR+RT program in Ovx rats led to lower body mass gain, liver triacylglycerol (TAG) levels, and fat mass gain compared to sedentary normally fed Ovx rats ($P < 0.01$). Stopping both FR and RT over a 5-week period resulted in the regain of body weight, intra-abdominal fat pad weight and liver TAG ($P < 0.01$). When only FR was maintained, the regain of body and fat pad weight as well as liver and plasma TAG concentrations was completely prevented. However, when only RT was maintained, regain in the aforementioned parameters was attenuated but not prevented ($P < 0.05$).

Conclusion: It is concluded that following a FR+RT weight loss program, continuation of only RT constitutes an asset to attenuate body weight and fat mass regain in Ovx rats; although the impact is less than the maintaining FR alone. These results suggest that, in post-menopausal women, RT is a positive strategy to reduce body weight and fat mass relapse.

Key words: Food restriction, exercise, liver triacylglycerols, plasma lipid profile, body weight relapse.

1. Introduction

Menopause in women is associated with an increased risk of incidence of several deleterious metabolic effects which may compromise quality of life. There is clear evidence that the prevalence of all individual components of the metabolic syndrome increases in women after menopause [1]. The tendency for weight gain is one of the most frequently observed sign after menopause [2] and increases the risk of obesity-related diseases including coronary heart diseases and type 2 diabetes in post-menopausal women [3]. Although therapies aimed at preventing these changes in women include hormone replacement therapy (HRT) [4], the risks associated with HRT have prompted extensive studies for other preventive or therapeutic alternatives such as diet and exercise [5].

It is well established that ovariectomy (Ovx) in animals leads to increased food intake and body weight [6] thus resulting in increased adipose tissue and liver fat accretion [7,8]. The increase in liver fat infiltration is of special interest since recent evidences suggest that the deficiency in estrogens in rat directly contributes to liver fat accumulation [9,10]. Fatty liver is now considered as a hepatic component of the metabolic syndrome that is known to significantly enhance the risk of developing cardiovascular disease and/or type 2 diabetes [11]. Moreover, recent data suggest that ectopic fat in the liver may be more important than visceral fat in characterization of metabolically benign obesity in humans [12]. It is thus of importance to investigate non-pharmacological interventions that may influence the accumulation of adipose tissue and/or lipids in liver following estrogen withdrawal.

Resistance training (RT) may constitute an interesting adjunct to food restriction (FR) to control body weight and fat accumulation in post-menopausal women [13]. However, it is a common observation that long-lasting maintenance of weight loss is difficult to achieve and body weight relapse is a common feature [14-16]. We recently reported, using an animal model, that a FR+RT program was effective to attenuate the ovariectomy-induced increase in body fat, especially in liver [17]. In line with this approach, the present study investigates the effect of maintaining RT or FR on body weight

regain, fat mass, and liver lipid infiltration in estrogen deficient animals previously submitted to a FR+RT weight loss program.

2. Methods

2.1. Animal care

Female Sprague-Dawley strain rats (n = 64) weighing 180-200g (6 weeks of age) were obtained from Charles River (St-Constant, PQ, Canada) and were housed individually. The 12:12-h light-dark cycle started at 6:00 AM and the room temperature was maintained at 20-23°C. All rats received usual pellet rat chow, referred to as the standard diet (SD; 12.5% fat; 63.2% carbohydrate; 24.3% protein; kcal, Agribrands Purina Canada, Woodstock, ON) and had free access to tap water. The experiments described in this report were conducted according to the directives of the Canadian Council on Animal Care after institutional approval.

2.2. Surgery

Two days after their arrival to our laboratory, rats were randomly divided into ovariectomized (Ovx) and sham-operated (Sham) groups. Ovx was conducted according to the technique described by Robertson *et al.* [18]. Animals were injected with antibiotics (Tribrissen 24%; 0.125cc/kg, sc) for three days, beginning the day before surgery. Details of the surgery have been previously described [19]. Following surgery, body weight and food intake were monitored three times per week in all rats.

2.3. Groups, food restriction and training protocol

Rats were randomly divided into 8 groups (n = 8/group) that had similar initial mean body mass and were treated similarly in terms of daily manipulations. A schematic representation of the experimental design is presented in Fig. 1. Rats that had been ovariectomized for 5 weeks were first submitted to a program of weight loss consisting of a FR regimen combined with a program of RT (OvxFR+RT) for 8 weeks (weeks 6-13). Following this period, OvxFR+RT rats were sub-divided into 3 groups for an additional 5 weeks (weeks 14-18): One group stopped both RT and FR (Ovx[postFR-RT]), one group continued FR while stopping RT (Ovx[contFRw/oRT]), and the last group went back to a normal *ad libitum* feeding while continuing RT (Ovx[contRTw/oFR]). Subgroups of Sham

and Ovx rats were either sacrificed after 13 or 18 weeks. The FR program consisted in a target 25% daily caloric reduction calculated from the daily energy intake average of the 2 preceding wk in normally fed rats. It turned out that the daily caloric reduction was 26% for the rats submitted to this program. RT was conducted using a device similar to the one described by Murphy *et al.* [20]. The resistance training program consisted of climbing on a 1.5 m metal grill with a slope of 7° from the wall, 4 times a week with a progressively increasing load up to 50% of body weight attached to the tail. The climbing grid (0.6-cm mesh) was divided into six 15-cm lanes. The animals were placed at the bottom of the grid (approximately 60 cm from the floor), head up, and climbed to the top where they could rest on a platform. The number of repetitions increased progressively during the first 2 weeks from 2 sets of 10 repetitions to 4 sets of 10 repetitions. Each repetition lasted ~3-5 s with ~40 s rest between repetitions. Exercise animals were sacrificed 48 h after the last exercise session.

2.4. Sacrifice

Rats were sacrificed between 09:00 and 12:00 AM. Remaining food was removed from the animal's cage at least 3 h before sacrifice. Immediately after complete anesthesia (pentobarbital sodium; 50mg/kg, ip), the abdominal cavity was opened following the median line of the abdomen and approximately 4 mL of blood was collected from the abdominal vena cava (<45s) into syringes pre-treated with ethylenediaminetetraacetic acid (15%; EDTA). Blood was centrifuged (3000 rpm; 4°C; 12 min; Beckman GPR Centrifuge) and the plasma was kept for further analysis. Several organs and tissues were removed and weighed (Mettler AE 100) in the following order: liver, uterus, mesenteric, urogenital, retroperitoneal, and subcutaneous fat deposits along with 4 skeletal muscles of the right hindlimb (soleus, plantaris, medial gastrocnemius, and lateral gastrocnemius). All tissue samples were frozen in liquid nitrogen immediately after they were weighed. The liver median lobe was freeze-clamped and used for triacylglycerol (TAG) determination. Mesenteric fat was collected from the superficial area covering the alimentary tract, the spleen and the pancreas. Special care was taken to distinguish fat cells from pancreatic cells

based on color and texture differences. On the right side of the animal, the subcutaneous fat was removed from the region between the caudal border of the rib cage, the dorsal and ventral midlines of the body and the urogenital organs. All rats were visually inspected for presence or not of ovaries, and uteri were excised and weighed to confirm ovariectomy or sham surgery. All tissue and plasma samples were stored at -78° C until analyses were performed.

2.5. Analytical procedures

Plasma 17β-estradiol and insulin concentrations were determined with radioimmunoassay test kits distributed by ICN Biomedicals (Costa Mesa, CA) and MEDICORP Laboratories (Montreal, PQ, Canada) respectively. Plasma glucose concentrations were determined with the use of a glucose analyzer (Yellow Springs Instruments 2300, Yellow Springs, OH). Plasma glucose and insulin values were used to calculate a homeostasis model assessment of insulin resistance (HOMA-IR) as followed: glucose (mmol/l) x insulin (mIU/ml)/22.5 [21]. Plasma free fatty acid (FFA) and TAG concentrations were determined with an enzymatic colorimetric assay available from Roche Diagnostics (Mannheim, Germany) and SIGMA (Saint Louis, Missouri, USA), respectively. Liver TAG concentrations were estimated from glycerol released after ethanolic KOH hydrolysis by using commercial kit from SIGMA (Saint Louis, Missouri, USA).

2.6. Statistical analysis

Values are expressed as means ± S.E. Statistical analyses were performed by one-way ANOVA for non-repeated measures design using treatment as a main effect. These analyses were conducted separately at two different times (13 and 18 weeks). Fisher's post hoc test was used in the event of a significant ($P < 0.05$) F ratio. Ovx effects on body weight change and food intake between weeks 1 and 5 were analyzed using unpaired *t*-test.

3. Results

3.1. Final body weight, body weight change and food intake

Ovx resulted in higher body weight measured either after 13 and 18 weeks (Table 1). The FR+RT program significantly ($P < 0.01$) reduced final body weight in Ovx animals to the level measured in Sham rats. Stopping both FR and RT treatments between weeks 13 and 18 re-established body weight values back to those of Ovx animals (Table 1). This regain in body weight was, however, completely prevented by maintaining FR while stopping RT. Continuing only RT resulted in body weight values that were midway between those measured in corresponding Sham and Ovx animals. The extents of these body weight changes are illustrated in Fig. 2A. These data first show that the gain in body weight following Ovx was mainly observed during the first 5-week post-surgery. These data also show the attenuation of body weight regain when FR or RT was maintained.

Food intake measured throughout the experiment follows a pattern similar to body weight changes (Fig. 2B). Food intake was higher ($P < 0.01$) in Ovx than in Sham rats between weeks 1-5 but not between weeks 6-13 and weeks 14-18. As planned, the FR program resulted in a lower ($P < 0.01$) food intake in OvxFR+RT (weeks 6-13) and Ovx[contFRw/oRT] (weeks 14-18) compared to all other *ad libitum* fed animals. Abandoning FR also led to increased ($P < 0.01$) food intake (weeks 14-18).

3.2. Uterus weight, plasma estradiol, and sum of 4 leg muscles weight.

All Ovx animals showed lower ($P < 0.01$) uterus weight and plasma estradiol concentrations than Sham rats confirming total ovariectomy (Table 1). The sum of 4 muscles weight was significantly ($P < 0.01$) increased in Ovx compared to Sham rats. The FR+RT program, however, decreased ($P < 0.01$) muscle mass to the level measured in Sham rats (Table 1). The abandon of both of the FR+RT components along with the selective continuation of RT or FR maintained muscle mass to the level of Ovx animals.

3.3. Plasma glucose, insulin, and free fatty acids concentrations

Plasma glucose concentrations were higher in Ovx compared to almost all other groups (week 18) (Table 2). The FR+RT regimen between weeks 6-13 in OvxFR+RT and continuing either FR or RT (weeks 14-18) in Ovx[contFRw/oRT] and Ovx[contRTw/oFR], led to lower plasmatic concentrations of insulin compared to corresponding Ovx rats. On the other hand, stopping both FR and RT treatments between weeks 13 and 18 (Ovx[postFR-RT]) increased the plasma insulin concentrations to the levels observed in Ovx rats (week 18). Calculation of the HOMA-IR score indicates lower values following the FR+RT program and the maintenance of either FR or RT of this program. There were no inter-group differences in plasma free fatty acid levels at week 13, but Ovx[contFRw/oRT] rats had lower ($P < 0.05$) plasma FFA concentrations compared to Sham, Ovx, and Ovx[postFR-RT] animals at week 18 (Table 2).

3.4. Liver and plasma TAG concentrations

Ovx resulted in significantly ($P < 0.01$) higher levels of liver TAG measured either after 13 and 18 weeks (Fig. 3A). FR+RT between weeks 5 and 13 significantly ($P < 0.01$) reduced liver lipid infiltration in Ovx rats to the levels of Sham rats. Abandoning both FR and RT between weeks 13 and 18 resulted in an increase in liver fat to the levels measured in Ovx animals. Interestingly, continuing either FR or RT kept liver TAG levels to the level measured in Sham rats.

Plasma TAG levels were not significantly different between Ovx and Sham rats after 13 weeks but were lower in Ovx than in Sham after 18 weeks (Fig. 3B). The FR+RT weight loss program reduced ($P < 0.05$) plasma TAG accumulation in Ovx rats (week 13). Plasma TAG concentrations returned to higher levels than normally fed Ovx rats ($P < 0.05$) 5 weeks after interrupting both FR and RT between weeks 13 and 18. Similarly to liver TAG, continuing either FR or RT prevented the increase in plasma TAG (week 18).

3.5. Intra-abdominal and subcutaneous fat pads

The sum of 3 intra-abdominal (mesenteric, urogenital, and retroperitoneal) fat pads weight and subcutaneous fat depots were higher in Ovx than in Sham rats either after 13 or 18 weeks (Fig. 4). FR+RT significantly ($P < 0.01$) reduced fat depots in Ovx rats. Stopping FR+RT in Ovx animals during 5 weeks resulted in a pronounced regain of fat to the levels of normally fed Ovx rats. On the other hand, maintenance of FR or RT largely prevented the fat regain although this effect was more pronounced when FR was maintained.

4. Discussion

Weight loss achieved through lifestyle interventions such as diet and exercise may be beneficial in reducing the risks of developing obesity and associated metabolic disorders in post-menopausal women [22,23]. Several strategies have been put forward to improve maintenance of weight loss in which, most involve changes in diet and increased levels of physical activity [24-26]. In recent years, our research group used an ovariectomized rat model to show that the addition of RT to a FR treatment synergistically reduced abdominal fat deposition and completely prevented liver lipid infiltration [17]. Results of the present study confirm the efficiency of the FR+RT program in reducing body weight as well as liver and adipocytes fat accretion in Ovx rats. The efficiency of the present FR+RT program is further supported by the subsequent relapse in all measured metabolic parameters in rats that were removed from the program for 5 weeks. In a recent study [27] we also observed that RT may be successfully substituted to a FR regimen to avoid body weight and fat mass regain. In line with this, the present study sought to determine if keeping the RT component of the FR+RT weight loss program would be successful in avoiding the relapse of body weight and fat mass. Results of the present study indicate that, although maintaining FR was more efficient than maintaining RT alone, the latter was successful in reducing the relapse in body weight, fat mass, and plasma TAG concentrations.

Liver lipid infiltration is known to increase in Ovx rats [28] and to a certain extend in post-menopausal women [29]. The present effect of RT on preventing liver TAG re-increase follows a pattern similar to that found for intra-abdominal fat accumulation. Both of these responses could, therefore, be simply associated with increased energy expenditure with the maintenance of the RT program. However, in recent studies, we reported observations suggesting that liver fat accumulation in Ovx animals may be regulated differently than adipocytes fat accumulation. For instance, we observed that adding RT to a program of FR is more successful in preventing fat accumulation in liver than FR alone [17]. These observations are in line with recent findings that the absence of estrogens alters

gene expressions in liver favoring a reduction in lipid oxidation and an increase in lipogenesis [10]. We previously suggested that RT may specifically regulate the levels of fat accumulation in liver by, for instance, maintaining the level of fat oxidation in spite of estrogen deficiency [17]. Although the present study does not provide any molecular and/or enzymatic evidence to support this interpretation, it does indicate that liver fat accumulation in Ovx rats is not solely regulated by an increased food intake.

Although maintenance of RT attenuated the relapse of body weight and fat accumulation in the present study, body weight, and fat mass regain was better preserved with the maintenance of the FR regimen. This indicates that the FR component of the FR+RT program is more important than the RT component. Nevertheless, the contribution of the RT component cannot be considered as non-significant since its maintenance attenuated the relapse. In a recent study [27], we observed that substituting FR by RT resulted in a large attenuation of body weight and fat regain. This suggests that a RT program might have a larger impact on maintaining body weight and fat loss when it is newly introduced into a program of weight loss than when it is already part of a FR weight loss program. It is likely that the biological interactions between FR and RT upon reducing body weight and fat accumulation are dominated by the FR intervention. Cessation of FR would therefore have a larger impact on fat regain than training cessation, as observed in the present study. This interpretation means that the impact of a RT program on body weight and fat mass reduction and/or maintenance is different when pursued in conjunction or in substitution of a FR program. Although identification of regulatory mechanisms are beyond the aim of the present study, it is possible on a speculative point of view, that gene expression and activity of key enzymes involved in metabolic pathways that may have an impact on body weight and fat accumulation are activated to a different extent whether RT is conducted simultaneously or substituted to a FR regimen.

Exercise training and FR may both affect peripheral insulin sensitivity. Although the rats in the present study were not in an overnight fasted state (3 h fast or more), we calculated HOMA-IR to obtain an index of insulin sensitivity. This index indicates a

deterioration of insulin sensitivity with Ovx, which was improved by the FR+RT program. Interestingly, maintenance of either FR or RT contributed similarly to the maintenance of low levels of HOMA-IR score. Although insulin sensitivity in the present context should be measured using more sophisticated techniques, the present data suggest that RT may be an asset in maintaining gain in insulin sensitivity after withdrawal of ovarian hormones.

As previously reported, Ovx resulted in an increase in muscle mass most likely secondary to an increase in body weight [17,30]. Accordingly, muscle mass was significantly decreased following the FR+RT program and re-increased after the total abandon of the program. It is likely, as it is the case for body weight and fat mass accumulation, that these changes in muscle mass are related more to the FR than to the RT component of the weight loss program. The incapacity of the RT program to maintain muscle mass when combined to FR program might be due to the substantial food restriction (26%) used in the present study. It is difficult to determine the real impact of the RT program on muscle mass in this study due to the confounding effect of the FR program.

In summary, results from the present study show that body weight and fat mass regain after the cessation of a weight loss program consisting of FR and RT in Ovx rats is minimized by the maintenance of FR regimen. Maintenance of RT program also constitutes an asset to attenuate body weight and fat mass regain, although the impact is less than maintaining FR alone. On a clinical point of view, the present results suggest that the maintenance of only one component of a FR+RT weight loss program constitutes a positive strategy to reduce body weight and fat mass relapse in post-menopausal women.

Acknowledgments

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References

[1] Alexander J, Clearfield M. Cardiovascular disease after menopause: a growing epidemic. Minerva Ginecol 2006;58:35-40.

[2] von Muhlen DG, Kritz-Silverstein D, Barrett-Connor E. A community-based study of menopause symptoms and estrogen replacement in older women. Maturitas 1995;22:71-8.

[3] Haffner SM, Katz MS, Dunn JF. Increased upper body and overall adiposity is associated with decreased sex hormone binding globulin in postmenopausal women. Int J Obes 1991;15(7):471-8.

[4] Tchernof A, Calles-Escandon J, Sites CK, Poehlman ET. Menopause, central body fatness, and insulin resistance: effects of hormone-replacement therapy. Coron Artery Dis 1998;9:503-11.

[5] Siddiqui NI, Rahman S, Mia AR, Shamsuzzaman AK. Evaluation of hormone replacement therapy. Mymensingh Med J 2005;14(2):212-8.

[6] Latour MG, Shinoda M, Lavoie J-M. Metabolic effects of physical training in ovariectomized and hyperestrogenic rats. J Appl Physiol 2001;90:235-41.

[7] Deshaies Y, Dagnault A, Lalonde J, Richard D. Interaction of corticosterone and gonadal steroids on lipid deposition in the female rat. Am J Physiol 1997;273:E355-62.

[8] Picard F, Deshaies Y, Lalonde J, et al. Effects of the estrogen antagonist EM-652.HCl on energy balance and lipid metabolism in ovariectomized rats. Int J Obes Relat Metab Disord 2000;24:830-40.

[9] Barsalani R, Pighon A, Rabasa-Lhoret R, Yasari S, Lavoie J-M. Liver of ovariectomized rats is resistant to resorption of lipids. Physiol Behav 2008;95:216-21.

[10] Paquette A, Wang D, Jankowski M, Gutkowska J, Lavoie J-M. Effects of ovariectomy on PPARalpha, SREBP-1c, and SCD-1 gene expression in the rat liver. Menopause 2008;15: In Press, DOI:10.1097/gme.0b013c31817b8159..

[11] Schneider JG, Tompkins C, Blumenthal RS, Mora S. The metabolic syndrome in women. Cardiol Rev 2006;14:286-91.

[12] Stefan N, Kantartzis K, Machann J, et al. Identification and characterization of metabolically benign obesity in humans. Arch Intern Med 2008;168(15):1609-16.

[13] Ryan AS, Pratley RE, Elahi D, Goldberg AP. Changes in plasma leptin and insulin action with resistive training in postmenopausal women. Int J Obes Relat Metab Disord 2000;24:27-32.

[14] Westerterp-Plantenga MS, Kempen KP, Saris WH. Determinants of weight maintenance in women after diet-induced weight reduction. Int J Obes Relat Metab Disord 1998;22:1-6.

[15] Pasman WJ, Saris WH, Westerterp-Plantenga MS. Predictors of weight maintenance. Obes Res 1999;7:43-50.

[16] Glenny AM, O'Meara S, Melville A, Sheldon TA, Wilson C. The treatment and prevention of obesity: a systematic review of the literature. Int J Obes Relat Metab Disord 1997;21:715-37.

[17] Corriveau P, Paquette A, Brochu M, Prud'homme D, Rabasa-Lhoret R, Lavoie J-M. Resistance training prevents liver fat accumulation in ovariectomized rats. Maturitas 2008;59:259-67.

[18] Robertson MC, Owens RE, Klindt J, Friesen HG. Ovariectomy leads to a rapid increase in rat placental lactogen secretion. Endocrinology 1984;114:1805-11.

[19] Shinoda M, Latour MG, Lavoie J-M. Effects of physical training on body composition and organ weights in ovariectomized and hyperestrogenic rats. Int J Obes Relat Metab Disord 2002;26:335-43.

[20] Murphy RJ, Beliveau L, Seburn KL, Gardiner PF. Clenbuterol has a greater influence on untrained than on previously trained skeletal muscle in rats. Eur J Appl Physiol Occup Physiol 1996;73:304-10.

[21] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9.

[22] Park HS, Lee KU. Postmenopausal women lose less visceral adipose tissue during a weight reduction program. Menopause 2003;10:222-7.

[23] Turcato E, Zamboni M, De Pergola G, et al. Interrelationships between weight loss, body fat distribution and sex hormones in pre- and postmenopausal obese women. J Intern Med 1997;241:363-72.

[24] Leermakers EA, Perri MG, Shigaki CL, Fuller PR. Effects of exercise-focused versus weight-focused maintenance programs on the management of obesity. Addict Behav 1999;24:219-27.

[25] Svendsen OL, Hassager C, Christiansen C. Six months' follow-up on exercise added to a short-term diet in overweight postmenopausal women--effects on body composition, resting metabolic rate, cardiovascular risk factors and bone. Int J Obes Relat Metab Disord 1994;18:692-8.

[26] Andersen RE, Wadden TA, Bartlett SJ, Zemel B, Verde TJ, Franckowiak SC. Effects of lifestyle activity vs structured aerobic exercise in obese women: a randomized trial. JAMA 1999;281:335-40.

[27] Pighon A, Paquette A, Barsalani R, et al. Substititing food restriction by resistance training prevents liver and adipocyte fat regain in Ovx rats. Climacteric 2008 In press.

[28] Paquette A, Shinoda M, Rabasa Lhoret R, Prud'homme D, Lavoie J-M. Time course of liver lipid infiltration in ovariectomized rats: impact of a high-fat diet. Maturitas 2007;58:182-90.

[29] Clark JM. Weight loss as a treatment for nonalcoholic fatty liver disease. J Clin Gastroenterol 2006;40:S39-43.

[30] Richard D. Effects of ovarian hormones on energy balance and brown adipose tissue thermogenesis. Am J Physiol 1986;250:R245-9.

Table 1. Final body weight (Wt.), uterus Wt., plasma estradiol, and sum of 4 leg muscles Wt. measured after 13 and 18 weeks.

Week	Sham	Ovx	OvxFR+RT	Ovx[postFR-RT]	Ovx[contFR w/oRT]	Ovx[contRT w/oFR]
Final body Wt. (g)						
13	310 ± 10§§	379 ± 11	300 ± 7§§			
18	336 ± 9§§,**,bb	411 ± 10aa		392 ± 10aa	327 ± 6	368 ± 13§§,aa
Uterus Wt. (mg)						
13	681 ± 63§§,++	104 ± 5	110 ± 7			
18	635 ± 64§§,**,aa,bb	102 ± 6		94 ± 3	88 ± 3	103 ± 5
Estradiol (pg/ml)						
13	157 ± 23§§,++	72 ± 4	62 ± 6			
18	121 ± 17§§,**,aa,bb	67 ± 4		70 ± 4	61 ± 4	62 ± 3
Sum of 4 leg muscles Wt. (g)						
13	2.1 ± 0.06§§	2.5 ± 0.07++	2.2 ± 0.08§§			
18	2.1 ± 0.04§§,**,a,bb	2.5 ± 0.11		2.5 ± 0.09	2.3 ± 0.07	2.5 ± 0.08

Values are mean ± SE. Sham: sham-operated; Ovx: ovariectomized; FR: food restriction; RT: resistance training; cont: continued; w/o: without. 5 week Ovx rats were first submitted to a program of weight loss consisting of a FR regimen combined with a program of RT (OvxFR+RT) for 8 weeks (weeks 6–13). Following this period, OvxFR+RT rats were sub-divided into 3 groups for an additional 5weeks (weeks 14–18): One group stopped both RT and FR (Ovx[postFR-RT]), one group stopped only RT (Ovx[contFRw/oRT]), and the last group stopped only FR (Ovx[contRTw/oFR]).

[a] Significantly different from Ovx[contFRw/oRT], $P < 0.05$.

[aa] $P < 0.01$.

[bb] Significantly different from Ovx[contRTw/oFR], $P < 0.01$.

** Significantly different from Ovx[postFR-RT], $P < 0.01$.

§§ Significantly different from Ovx, $P < 0.01$.

++ Significantly different from OvxFR+RT, $P < 0.01$.

Table 2. Plasma concentrations of glucose, insulin, HOMA-IR, and free fatty acids (FFA) measured after 13 and 18 weeks.

Week	Sham	Ovx	OvxFR+RT	Ovx[postFR-RT]	Ovx[contFR w/oRT]	Ovx[contRT w/oFR]
Glucose (mM)						
13	8.6 ± 0.4	9.3 ± 0.3	9.8 ± 0.4			
18	8.0 ± 0.1§§	9.8 ± 0.4		8.6 ± 0.3§	8.8 ± 0.4	8.3 ± 0.4§§
Insulin (pM)						
13	403 ± 42§	568 ± 45	304 ± 36§§			
18	430 ± 43aa,bb	434 ± 26		426 ± 28	237 ± 40§§,**	256 ± 23§§,**
HOMA-IR						
13	25.7 ± 2.9§§	37.2 ± 3.5	22.3 ± 3.1§§			
18	25.8 ± 2.7§§,aa,bb	33.3 ± 2.4		27.5 ± 2.3	15.5 ± 3.2§§,**	15.7 ± 3.1§§,**
FFA (mM)						
13	0.093 ± 0.01	0.073 ± 0.01	0.066 ± 0.02			
18	0.125 ± 0.02b	0.101 ± 0.02		0.107 ± 0.02	0.049 ± 0.01§,*	0.081 ± 0.02

Values are mean ± SE. HOMA-IR: homeostatic model assessment of insulin resistance [(glucose: mmol/l × insulin: mlU/ml)/22.5] (3 h fasted state). Abbreviations and groups are described in Table 1.

aa Significantly different from Ovx[contFRw/oRT], $P < 0.01$.

b Significantly different from Ovx[contRTw/oFR], $P < 0.05$.

bb $P < 0.01$.

§ Significantly different from Ovx, $P < 0.05$.

§§ $P < 0.01$.

* Significantly different from Ovx[postFR-RT], $P < 0.05$.

** $P < 0.01$.

Legends

Fig. 1. Schematic representation of the experimental design. The tip of the arrow indicates the time of sacrifice. Sham: sham-operated, Ovx: ovariectomized, FR: food restriction, and RT: resistance training.

Fig. 2. Body weight change (A) and average daily food intake (B) during weeks 0–5, weeks 6–13, and weeks 14–18. Values are mean ± SE. §§ Significantly different from Ovx, $P < 0.01$. ++Significantly different from OvxFR+RT, $P < 0.01$. *Significantly different from Ovx[postFR-RT], $P < 0.05$, **$P < 0.01$. ^{aa}: Significantly different from Ovx[contFRw/oRT], $P < 0.01$. ^{bb}: Significantly different from Ovx[contRTw/oFR], $P < 0.01$. Abbreviations and groups are described in Table 1.

Fig. 3. Liver triacylglycerol (TAG) concentration (A) and plasma concentration of TAG (B) measured after 13 and 18weeks. Values are mean ± SE. § Significantly different from Ovx, $P < 0.05$, §§$P < 0.01$. +Significantly different from OvxFR+RT, $P < 0.05$, ++$P < 0.01$. *Significantly different from Ovx[postFR-RT], $P < 0.05$, **$P < 0.01$. ^{aa}: Significantly different from Ovx[contFRw/oRT], $P < 0.01$. ^{bb}: Significantly different from Ovx[contRTw/oFR], $P < 0.01$. Abbreviations and groups are described in Table 1.

Fig. 4. Sum of 3 intra-abdominal (mesenteric, urogenital, and retroperitoneal) fat depots weight (A) and sub-cutaneous fat pad weight (B) measured after 13 and 18 weeks. Values are mean ± SE. § Significantly different from Ovx, $P < 0.05$, §§$P < 0.01$. ++Significantly different from OvxFR + RT, $P < 0.01$. **Significantly different from Ovx[postFR-RT], $P < 0.01$. ^{a}: Significantly different from Ovx[contFRw/oRT], $P < 0.05$,

[aa]: $P < 0.01$. [bb]: Significantly different from Ovx[contRTw/oFR], $P < 0.01$. Abbreviations and groups are described in Table 1.

Fig. 1.

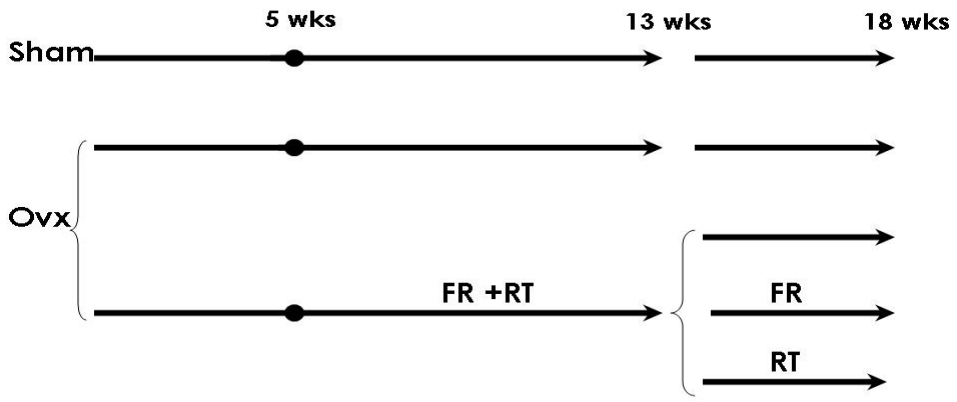


Fig. 2.

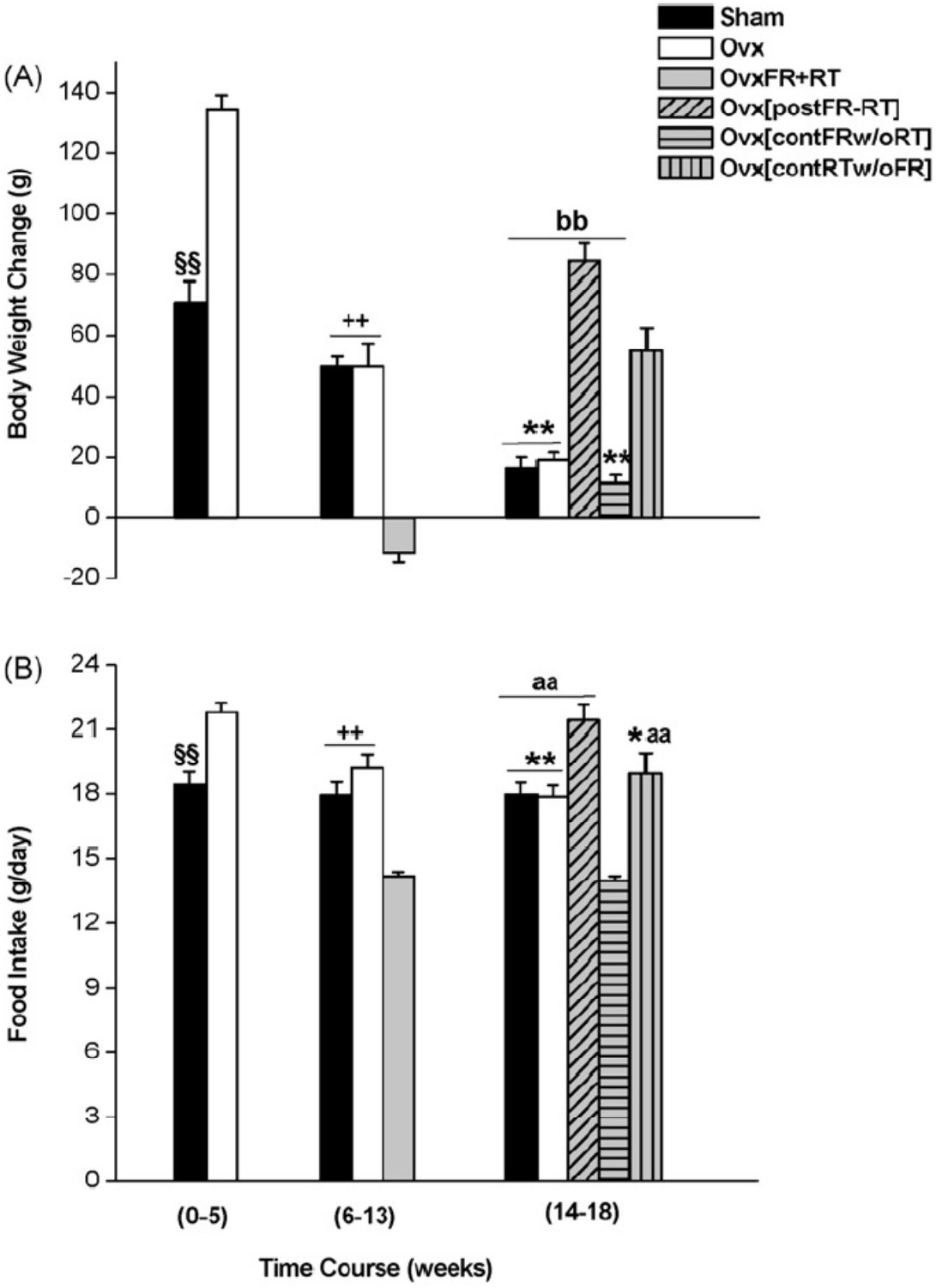

Sham
Ovx
OvxFR+RT
Ovx[postFR-RT]
Ovx[contFRw/oRT]
Ovx[contRTw/oFR]
(A)
Body Weight Change (g)
140
120
100
80
60
40
20
0
-20
§§
++
bb
**
**
(B)
Food Intake (g/day)
24
21
18
15
12
9
6
3
0
§§
++
aa
**
*aa
(0-5)
(6-13)
(14-18)
Time Course (weeks)

Fig. 3.

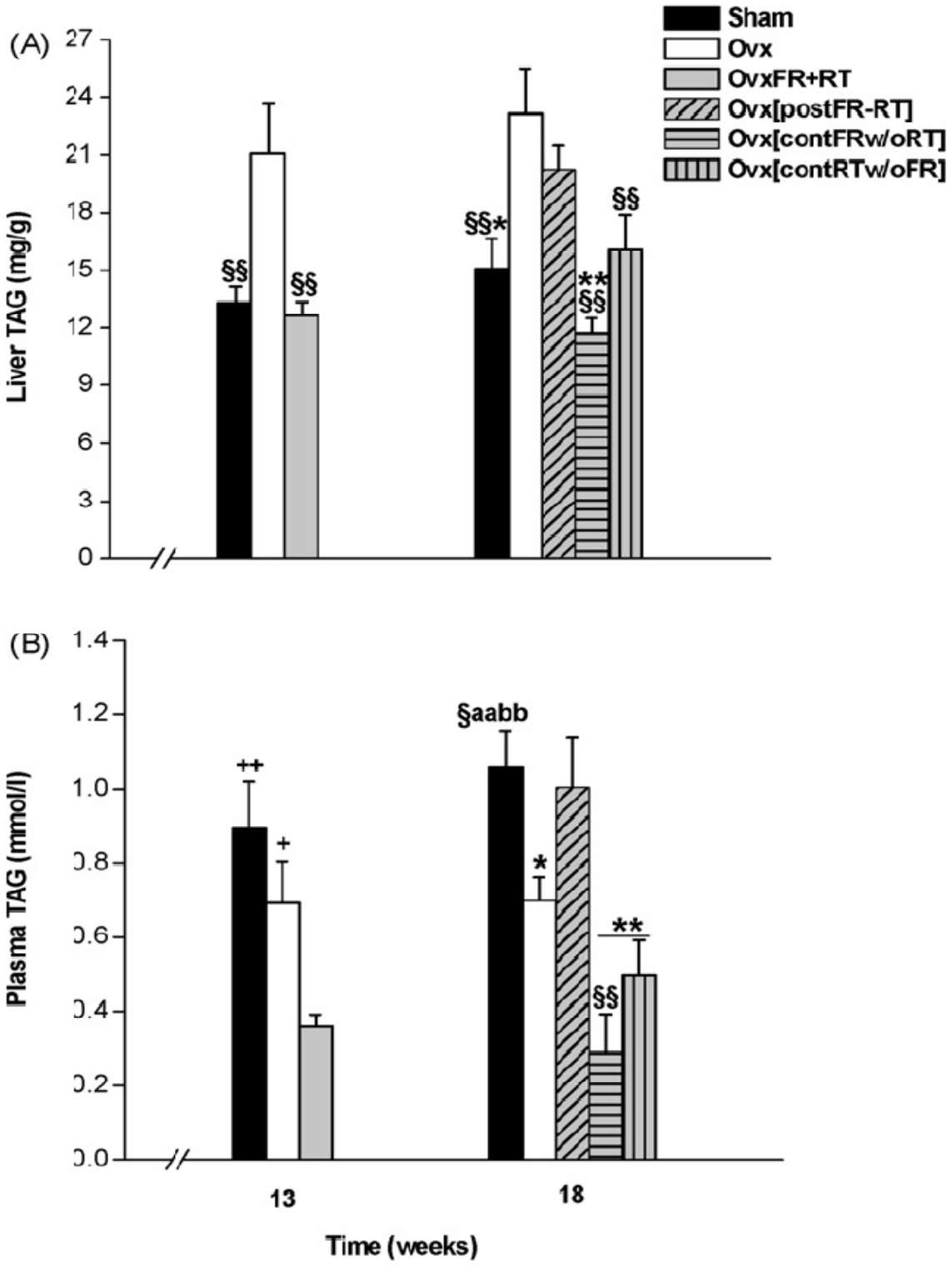
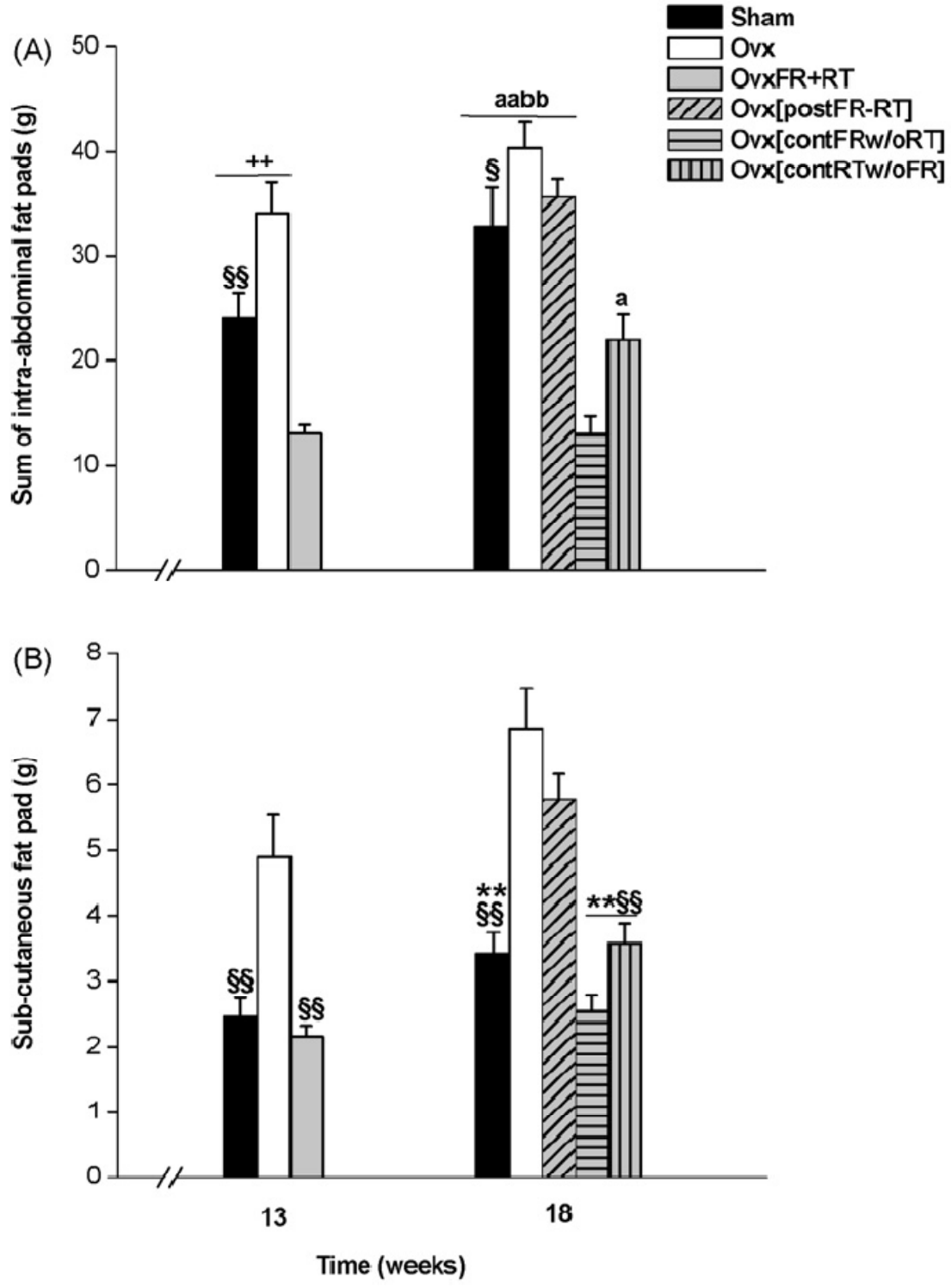

(A)
Liver TAG (mg/g)
27
24
21
18
15
12
9
6
3
0
§§
§§
§§*
**
§§
§§
Sham
Ovx
OvxFR+RT
Ovx[postFR-RT]
Ovx[contFRw/oRT]
Ovx[contRTw/oFR]
(B)
Plasma TAG (mmol/l)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
++
+
§aabb
*
**
§§
13
18
Time (weeks)

Fig. 4.


Sham
Ovx
OvxFR+RT
Ovx[postFR-RT]
Ovx[contFRw/oRT]
Ovx[contRTw/oFR]
(A)
Sum of intra-abdominal fat pads (g)
50
40
30
20
10
0
++
§§
§
aabb
a
(B)
Sub-cutaneous fat pad (g)
8
7
6
5
4
3
2
1
0
§§
§§
**
§§
**§§
13
18
Time (weeks)

Chapter 4: Original article 3

Title:

Does exercise training prior to Ovx protect against liver and adipocyte fat accumulation in rats?

Authors:

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Does exercise training prior to Ovx protect against liver and adipocyte fat accumulation in rats?

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Short title: Ovariectomy in exercise trained rats.

Key words: Hepatic steatosis, Plasma lipid profile, HOMA-IR, Estrogens, Training cessation.

ABSTRACT

Objective: To determine whether a training state protects against the metabolically deleterious effects of ovariectomy on liver and adipocytes fat accumulation in rats.

Design: Female rats were randomly assigned to each group (n = 8 rats/group). The animals were first either exercise trained (Tr) for 6 weeks or kept sedentary (Sed) before being sham operated (Sham), ovariectomized (Ovx), or Ovx with 17 β-estradiol supplementation (OvxE2). Following surgery, sedentary rats either remained sedentary (Sed-Sed) or undertook exercise training for 6 weeks (Sed-Tr) while exercise-trained rats either became sedentary (Tr-Sed) or resumed exercise training (Tr-Tr).

Results: Body weight and energy intake along with intra-abdominal and subcutaneous fat pad weights and homeostasis model assessment of insulin resistance (HOMA-IR) were significantly ($P < 0.01$) increased in the Ovx group compared to the Sham and OvxE2 groups. Rats kept in a sedentary state after surgery showed the higher ($P < 0.05$) values for all of these variables whether they were trained or not before surgery (Sed-Sed and Tr-Sed), indicating no protective effect of a previous exercise-trained state. On the other hand, training conducted after surgery resulted in a lowering of fat mass and HOMA-IR whether rats had been trained or not before surgery (Sed-Tr and Tr-Tr), indicating the effectiveness of exercise training even initiated after surgery. These responses were independent of surgery. Interestingly, liver triacylglycerol concentrations followed a pattern of responses identical to fat mass with the exception that all of the responses were observed only in the Ovx group ($P < 0.05$).

Conclusion: There is no protective effect of a previous exercise-training state on ovariectomy-induced liver and adipocytes fat accumulation if rats remain sedentary after ovariectomy. However, training conducted concurrently with estrogen withdrawal has protective effects, especially on liver fat accumulation, whether or not rats were previously trained.

INTRODUCTION

Cardiovascular disease risk factors, such as lipid profile deterioration, become more pronounced after menopause,[1] making coronary heart disease a leading cause of death among postmenopausal women.[2] A large proportion of women after menopause gain weight, especially in the abdominal region, resulting in several metabolic disturbances.[3,4](for a review, see reference [5]) Recent evidence also suggests that menopause is associated with the development of a state of hepatic steatosis.[6,7] Excessive fat accumulation in hepatocytes has been shown to play an important role in the development of insulin resistance[8] and is even considered as a hepatic component of the metabolic syndrome.[9,10] The importance of the phenomenon is highlighted by recent data suggesting that ectopic fat in liver may be even more important than visceral fat in characterization of metabolically benign obesity in humans.[11] Liver fat accumulation with estrogen withdrawal is also well documented in animals in which ovariectomy leads to increased adipose tissue and liver fat accretion.[12-15] Considering the consequences of ectopic fat accumulation in liver, there is an important need to establish strategies to counteract this effect in postmenopausal women.

Although results from animal and human studies have shown a decrease in body weight and abdominal fat accumulation following estrogen replacement,[16-18] long-term estrogen supplementation in postmenopausal women could increase several health risks, such as cancer, and their utilization is still under debate.[19,20] Lifestyle modifications (diet and physical activity), therefore, constitute an interesting alternative to circumvent metabolic problems arising with menopause. Cross-sectional and randomized controlled trials studies indicate that physical activity can be an effective intervention to improve body fat and/or metabolic risks variables in overweight/obese postmenopausal women.[21-23] On the other hand, recent reviews[6,24] of lifestyle modifications on liver lipid infiltration in humans indicate a paucity of specific information on liver lipid infiltration associated with menopause. In animals, we recently reported that resistance training prevents liver fat accumulation in ovariectomized rats.[12,25] There is, however, no information to our

knowledge on the effects of an endurance training program on the prevention of liver fat accumulation with estrogen withdrawal.

An interesting question related to exercise and estrogen withdrawal is whether women who exercise regularly during their reproductive period are protected against the deleterious metabolic effects of menopause. In the only study conducted in women that addressed this question, cross-sectional comparisons indicated that there was no difference in percent body fat between premenopausal and postmenopausal trained runners.[26] There are, however, no randomized controlled trial studies on the effects of exercise on body fat and abdominal fat throughout menopause that would allow a firm conclusion on whether physical activity may prevent or limit the gain of total fat and abdominal fat during menopause. In the present study, we addressed this question using an animal model that allowed us to test a complete design of trained and untrained animals before and after withdrawal of estrogens.

METHODS

Animal care

Female Sprague-Dawley strain rats weighing 180-200 g (8-week-old) were obtained from Charles River (St-Constant, PQ, Canada) and were housed individually. The 12 : 12-h light-dark cycle started at 06:00 and the room temperature was maintained at 20-23°C. All rats received usual pellet rat chow, referred to as the standard diet (SD; 12.5% fat; 63.2% carbohydrate; 24.3% protein; kcal, Agribrands Purina Canada, Woodstock, ON) and had free access to tap water. Rats were randomly sequentially assigned to each group (n = 8 rats/group) that had initial similar mean body mass and were treated similarly in terms of daily manipulations. The experiments described in this report were conducted according to the directives of the Canadian Council on Animal Care after institutional approval.

Groups, surgery and post-surgery treatment

A schematic representation of the experimental design is presented in Figure 1. Rats were first submitted to an endurance training (Tr) program, or remained sedentary (Sed) for 6 weeks. Exercise training consisted of continuous running on a motor-driven rodent treadmill (Quinton Instruments, Seattle, WA), 5 times/week. The rats progressively ran 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. At the end of this first 6-week period, two groups (n = 8/group) of Sed and Tr animals were sacrificed. All other rats were ovariectomized (Ovx), sham-operated (Sham), or Ovx with 17β-estradiol supplementation (OvxE2).

Ovariectomy was conducted according to the technique described by Robertson *et al*.[27] Animals were injected with antibiotics (Tribrissen 24%; 0.125cc/kg, subcutaneously) for 3 days, beginning the day before surgery. For surgery, rats were anesthetized with a mixture of ketamine-xylazine (61.5-7.6 mg/kg, intraperitoneally). In OvxE2 rats, a small 17β-estradiol pellet (0.72 mg; 0.012 mg/d) with a biodegradable carrier binder efficient for 60 days (catalog no. SE-121; Innovative Research of America, Sarasota, FL) was placed subcutaneously between the shoulder blades. El-Mas and Abdel-Rahman[28] previously

showed that this estrogen regimen produces physiological levels of the hormone. A placebo 60-day pellet containing the binding carrier only was used in all other rats (catalog no. SC-111).

One week (7^{th} week) was provided for surgery and recovery in all rats. During this time, all rats were only submitted to a habituation running protocol (10-15 min/day) in the last 3 days. Thereafter, sedentary rats either remained sedentary (Sed-Sed) or started training (Sed-Tr) after surgery and trained rats either stopped (Tr-Sed) or resumed training (Tr-Tr) after surgery (Figure 1). Training resumed for rats assigned to training groups the next week for 5 more weeks. This second program of training was adjusted to be the same for all training rats and to be close to the training program used before surgery. The post-surgery exercise-trained rats progressively ran from 15 min/day at 15 m/min, 0% slope, up to 60 min/day at 26 m/min, 10% slope, for the last 3 weeks. Body weight and food intake were monitored every other day. Rats were sacrificed at the end of this second treatment (week 12). Exercise animals were sacrificed 48 h after the last exercise bout.

Blood and tissue sampling

Rats were sacrificed between 09:00 and 12:00. Remaining food was removed from the animal's cage at least 3 h before sacrifice. Immediately after complete anesthesia (pentobarbital sodium; 50mg/kg, intraperitoneally), the abdominal cavity was opened following the median line of the abdomen and approximately 4 ml of blood was collected from the abdominal vena cava (<45s) into syringes pre-treated with ethylenediaminetetraacetic acid (15%; EDTA). Blood was centrifuged (3000 rpm; 4°C; 12 min; Beckman GPR Centrifuge) and the plasma was kept for further analysis. Several organs and tissues were removed and weighed (Mettler AE 100) in the following order: liver, uterus, mesenteric, urogenital, retroperitoneal, and subcutaneous fat deposits along with four skeletal muscles of the right hind limb (soleus, plantaris, medial gastrocnemius, and lateral gastrocnemius). All tissue samples were frozen in liquid nitrogen immediately after they were weighed. The liver median lobe was freeze-clamped and used for triacylglycerol (TAG) determination. Mesenteric fat pad consisted of adipose tissue

surrounding the gastro-intestinal tract from the gastro-oesophageal 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 as well as ovaries, oviducts and uterus. Retroperitoneal fat pad was taken as that distinct deposit behind each kidney along the lumbar muscles. For subcutaneous fat deposit measurement, a rectangular piece of skin was taken on the right side of each animal, from the median line of the abdomen to the spine and the right hip to the first rib as described by Krotkiewski and Bjorntorp.[29] All rats were visually inspected for presence or not of ovaries, and uteri were excised and weighed to confirm ovariectomy or sham surgery. All tissue and plasma samples were stored at -78°C until analyses were performed.

Biochemical analyses

Plasma insulin concentrations were determined with radioimmunoassay test kit distributed by LINCO Research (St. Charles, Missouri, USA). Plasma glucose concentrations were determined with the use of a glucose analyzer (Yellow Springs Instruments 2300, Yellow Springs, OH). Plasma glucose and insulin values were used to calculate a homeostasis model assessment of insulin resistance (HOMA-IR) as follows: glucose (mmol/l) × insulin (mIU/L)/22.5.[30] Plasma free fatty acid (FFA) and TAG concentrations were determined with an enzymatic colorimetric assay available from Roche Diagnostics (Mannheim, Germany) and SIGMA (Saint Louis, Missouri, USA), respectively. Liver TAG concentrations were estimated from glycerol released after ethanolic KOH hydrolysis by using commercial kit from SIGMA (Saint Louis, Missouri, USA).

Statistical analysis

Values are expressed as means ± standard error. Statistical analyses were performed by two-way ANOVA for non-repeated measures design using surgery and training as main effects. Fisher's post hoc test was used in the event of a significant ($P < 0.05$) F ratio. Comparisons between Sed and Tr rats at week 6 were conducted using unpaired t-test.

RESULTS

All Ovx animals showed lower (P<0.01) uterus weight than Sham and OvxE2 rats, indicating total ovariectomy (Table 1). Moreover, uterus weight of OvxE2 rats was higher (P<0.01) compared to Sham rats, suggesting hyperestrogenic state in OvxE2 animals. The 6-week pre-surgery training increased (P<0.05) muscle weight compared to the weights in the sedentary group. Ovariectomy resulted in higher (P<0.01) muscle weight and plasma glucose concentrations compared to Sham and OvxE2 rats (Table 1). Estrogen replacement in OvxE2 led to lower plasma glucose concentrations than in Sham rats (P<0.01). There was no treatment effect on the sum of weights of the four leg muscles and plasma glucose concentrations.

Body weight (Figure 2a), energy intake (Figure 2b), intra-abdominal and subcutaneous fat pad weights (Figure 3), plasma insulin concentrations (Table 1), and HOMA-IR values (Figure 4) show a similar pattern of responses to the treatments. All of these parameters were significantly (P<0.01) increased in the Ovx compared to the Sham and OvxE2 groups. Body weight and HOMA-IR values were also significantly (P<0.01) reduced in OvxE2 compared to Sham rats (Fig. 2a and 4). Endurance exercise training conducted after the surgery decreased all of these variables in all groups whether rats have been trained or not before the surgery (Tr-Tr and Sed-Tr) (P<0.05). This also implies that rats kept sedentary after surgery had the higher values whether they were trained or not before surgery (Sed-Sed and Tr-Sed).

Liver TAG concentrations follow a similar response pattern to fat mass with the exception that the responses were seen only in the Ovx group (Figure 5). More specifically, Ovx-induced liver fat accumulation was reduced in trained rats independently of whether the rats were trained or not before surgery. As a rule, the higher values of liver fat accumulation were found if the rats that were kept sedentary after the ovariectomy, whether they were trained or not before surgery.

The only exceptions to the response patterns observed for all above-mentioned variables were plasma TAG and FFA. There were no effects of ovariectomy with and without E2 replacement on plasma TAG levels (Table 1). When rats were trained, either before or after surgery or both, plasma TAG concentrations show lower ($P<0.01$) values compared to rats continuously maintained in the sedentary state (Sed-Sed). On the other hand, the 6-week pre-surgery training decreased the plasma FFA levels ($P<0.01$) compared to the levels in sedentary animals (Table 1, week 6). Opposite to liver TAG, ovariectomy resulted in lower ($P<0.01$) plasma FFA concentrations than in Sham and OvxE2 in all treatment groups. Lower ($P<0.01$) plasma FFA values were found in Sed-Tr condition compared to Sed-Sed and Tr-Sed groups.

DISCUSSION

As previously reported[13,31,32] body weight, energy intake, adipose tissue mass, and muscles weights were higher in Ovx compared to Sham rats. These effects of ovariectomy were reversed in E2-supplemented rats. The present results also confirm recent observations that ovariectomy leads to liver TAG accretion in rats[12-15,33-35] that is prevented by E2 treatment.[13,35] E2 supplementation in Ovx rats has been reported to counteract the effects of estrogens withdrawal by lowering food intake, decreasing lipoprotein lipase activity, and increasing adipose tissue lipolysis and energy expenditure.[36] Similar to estrogen supplementation, the present results indicate that endurance exercise training conducted concurrently with the induction of ovariectomy significantly attenuated liver and adipocytes fat accumulation. However, an endurance exercise training state acquired before ovariectmoy does not provide any protective effects against ovariectmoy-induced fat accumulation if exercise is discontinued after the ovariectmoy.

Training before Ovx

Results of the present study indicate that there is no protective effect of a previous endurance exercise training state on ovariectmoy-induced liver and adipocytes fat accumulation in rats. The 6-week pre-surgery training resulted in decreased intra-abdominal fat and plasma FFA levels and increased muscle weight in comparison to animals kept sedentary, indicating that this endurance exercise training program was successful. In spite of this, fat accumulation in liver and adipocytes 6 weeks after ovariectmoy was increased to a similar extent in rats kept sedentary, whether they were trained or not before surgery (Sed-Sed vs Tr-Sed). The increased food intake observed in the Tr-Sed group to the level of the Sed-Sed group may have contributed to the higher fat accretion. Linked to this observation, it is important to consider that, in addition to the estrogen withdrawal, rats in the Tr-Sed group were also submitted to an exercise training cessation stimulus. Regular exercise has been reported to lower body weight and adiposity level in rats.[37] This has been shown to predispose to a rapid weight and fat regain upon cessation of a regular exercise training program.[38] Increased lipid storage after cessation of

exercise training has been attributed to multiple factors including increased tissue sensitivity to insulin, increased lipoprotein lipase activity, and reduced resting and exercise-induced energy expenditure.[39-42] In a previous study[38] it was found that endurance exercise training had no protective effect on fat accumulation following the initiation of a high-fat diet upon training cessation. Similar to these findings, the present study indicates that exercise training has no protective effect on fat accumulation following estrogens withdrawal upon training cessation. The only exception is the plasma TAG levels, which seem to be protected by a previous training state. Indeed, plasma TAG levels were lower in Tr-Sed than in Sed-Sed animals.

Training after Ovx

Training did have a significant metabolic impact when conducted concurrently with the induction of estrogens withdrawal. This first confirms what has been observed in previous animal studies,[12,25] that exercise training is a lifestyle modification that has an important metabolic impact to circumvent the deleterious effects of estrogen withdrawal. The present study adds to these reports by specifying that training must be pursued concurrently to the ovariectmoy to be effective. Although concurrent exercise training had a significant impact on fat mass in Ovx animals, it is important to consider that the effects of training on body weight, energy intake, fat mass, and HOMA-IR were not limited to the Ovx animals since Sham and OvxE2 rats also benefited from these adaptations if training was pursued between weeks 7 and 12. This suggests that training offers a protection against fat accumulation not only against estrogens withdrawal but also against fat accumulation in Sham and OvxE2 animals. In addition, endurance training initiated before the period of observation (weeks 0 to 6) did not seem to offer any supplementary protection since there were no significant differences between rats in Sed-Tr and Tr-Tr groups. However, the fact that rats had to stop training after surgery and slowly resumed training thereafter may explain the absence of additive effects of training in the Tr-Tr compared to the Sed-Tr rats. The present study was not aimed at studying the underlying mechanisms involved in the protective effect of endurance exercise training on fat accumulation in Ovx animals.

However, based on the present data, it is clear that the mechanisms involved in the training effects (i.e. increased energy expenditure) must be maintained to be effective.

The special case of liver fat accumulation with Ovx

One of the tissues that is particularly affected by fat accumulation in Ovx animals[12-15] as well as in postmenopausal women[6,7] is the liver. In the present study, the fact that liver fat accumulation was the only measured parameter in which the training and detraining status affected only the Ovx animals (not the Sham and OvxE2 groups), is of particular interest. Molecular and physiological results from recent studies conducted by our group indicated that liver lipid infiltration in Ovx animals may be related to reduced hepatic lipid oxidation and increased lipogenesis.[35,43] Results from these studies provided some support to the concept that estrogens act intrahepatically as a protective tool against liver lipid infiltration. Subsequently, we observed that lipid accumulation in liver following estrogen deprivation is hardly reversible by diet changes in this hormonal context, thus supporting the concept that fat accumulation in liver of Ovx animals is not only linked to increased energy intake.[34] In this context, it is particularly interesting to observe that training conducted concurrently with ovariectmoy largely reduced liver fat accumulation. This suggests that endurance exercise training could induce metabolic adaptations in liver similar to estrogen. Since liver fat accumulation has been shown to play an important role in the development of insulin resistance,[8] the present data support the importance of endurance exercise training to prevent liver lipid infiltration in postmenopausal women.

Metabolic consequences of fat accumulation in Ovx rats

To get an insight into the possibility that exercise training may overcome the metabolic consequences of fat accumulation in Ovx rats, we calculated the HOMA-IR as an index of insulin sensitivity. As previously observed,[12] ovariectomy was associated with a deterioration of insulin sensitivity. Although insulin sensitivity in the present context should be measured with more sophisticated techniques, it is revealing that training conducted concurrently with the induction of ovariectmoy resulted in the same pattern of positive effects as those observed for liver and adipocytes fat accumulation. This

observation is a further indication that training conducted concurrently with estrogen withdrawal is an asset not only to reduce liver and adipocytes fat accumulation, but also to attenuate the metabolic consequences associated with it.

In conclusion, results of the present study indicate that training has a significant impact on reducing fat accumulation in liver and adipocytes, as well as increasing insulin sensitivity based on HOMA-IR index, in Ovx rats if conducted concurrently with the initiation of the estrogen withdrawal. In addition, training conducted before the ovariectmoy does not provide any protection against the metabolic deleterious effects of ovariectmoy if endurance exercise training is not pursued after ovariectmoy. From a clinical point of view, these observations suggest that sedentary women who undertake an endurance exercise training program during the menopause transition will benefit from it even though they have not been training before. Although further clinical randomized studies in women are warranted, the present results also emphasize the recommendation that trained women should keep on training after menopause.

SOURCE OF FUNDING

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CONFLICT OF INTEREST

None.

REFERENCES

1. Hagey AR, Warren MP. Role of exercise and nutrition in menopause. *Clin Obstet Gynecol* 2008; 51:627-41.

2. Sharp PC, Konen JC. Women's cardiovascular health. *Prim Care* 1997; 24:1-14.

3. Bjorntorp P. Obesity and adipose tissue distribution as risk factors for the development of disease. A review. *Infusionstherapie* 1990; 17:24-7.

4. Folsom AR, Kushi LH, Anderson KE, Mink PJ, Olson JE, Hong CP, *et al.* Associations of general and abdominal obesity with multiple health outcomes in older women: the Iowa Women's Health Study. *Arch Intern Med* 2000; 160:2117-28.

5. Lobo RA. Metabolic syndrome after menopause and the role of hormones. *Maturitas* 2008; 60:10-8.

6. Clark JM. Weight loss as a treatment for nonalcoholic fatty liver disease. *J Clin Gastroenterol* 2006; 40 Suppl 1:S39-43.

7. Volzke H, Schwarz S, Baumeister SE, Wallaschofski H, Schwahn C, Grabe HJ, *et al.* Menopausal status and hepatic steatosis in a general female population. *Gut* 2007; 56:594-5.

8. Kadowaki T, Hara K, Yamauchi T, Terauchi Y, Tobe K, Nagai R. Molecular mechanism of insulin resistance and obesity. *Exp Biol Med (Maywood)* 2003; 228:1111-17.

9. Samuel VT, Liu ZX, Qu X, Elder BD, Bilz S, Befroy D, et al. Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. *J Biol Chem* 2004; 279:32345-53.

10. Marchesini G, Marzocchi R, Agostini F, Bugianesi E. Nonalcoholic fatty liver disease and the metabolic syndrome. *Curr Opin Lipidol* 2005; 16:421-7.

11. Stefan N, Kantartzis K, Machann J, Schick F, Thamer C, Rittig K, *et al.* Identification and characterization of metabolically benign obesity in humans. *Arch Intern Med* 2008; 168:1609-16.

12. Pighon A, Paquette A, Barsalani R, Chapados NA, Yasari S, Doucet E, *et al.* Substituting food restriction by resistance training prevents liver and body fat regain in ovariectomized rats. *Climacteric* 2009; 12(2):153-64.

13. Paquette A, Shinoda M, Rabasa Lhoret R, Prud'homme D, Lavoie JM. Time course of liver lipid infiltration in ovariectomized rats: impact of a high-fat diet. *Maturitas* 2007; 58:182-90.

14. Deshaies Y, Dagnault A, Lalonde J, Richard D. Interaction of corticosterone and gonadal steroids on lipid deposition in the female rat. *Am J Physiol* 1997; 273:E355-62.

15. Picard F, Deshaies Y, Lalonde J, Samson P, Labrie C, Belanger A, *et al.* Effects of the estrogen antagonist EM-652.HCl on energy balance and lipid metabolism in ovariectomized rats. *Int J Obes Relat Metab Disord* 2000; 24:830-40.

16. Tchernof A, Calles-Escandon J, Sites CK, Poehlman ET. Menopause, central body fatness, and insulin resistance: effects of hormone-replacement therapy. *Coron Artery Dis* 1998; 9:503-11.

17. Seidlova-Wuttke D, Hesse O, Jarry H, Christoffel V, Spengler B, Becker T, *et al.* Evidence for selective estrogen receptor modulator activity in a black cohosh (Cimicifuga racemosa) extract: comparison with estradiol-17beta. *Eur J Endocrinol* 2003; 149:351-62.

18. Seidlova-Wuttke D, Jarry H, Becker T, Christoffel V, Wuttke W. Pharmacology of Cimicifuga racemosa extract BNO 1055 in rats: bone, fat and uterus. *Maturitas* 2003; 44 Suppl 1:S39-50.

19. Recommendations for estrogen and progestogen used in peri-and postmenopausal women: October 2004 position statement of The North American Menopause Society. *Menopause* 2004; 11:589-600.

20. Hormone therapy for the prevention of chronic conditions in postmenopausal women: recommendations from the U.S. Preventive Services Task Force. *Ann Intern Med* 2005; 142:855-60.

21. Astrup A. Physical activity and weight gain and fat distribution changes with menopause: current evidence and research issues. *Med Sci Sports Exerc* 1999; 31:S564-7.

22. Frank LL, Sorensen BE, Yasui Y, Tworoger SS, Schwartz RS, Ulrich CM, *et al.* Effects of exercise on metabolic risk variables in overweight postmenopausal women: a randomized clinical trial. *Obes Res* 2005; 13:615-25.

23. Irwin ML, Yasui Y, Ulrich CM, Bowen D, Rudolph RE, Schwartz RS, *et al.* Effect of exercise on total and intra-abdominal body fat in postmenopausal women: a randomized controlled trial. *JAMA* 2003; 289:323-30.

24. Wang RT, Koretz RL, Yee HF, Jr. Is weight reduction an effective therapy for nonalcoholic fatty liver? A systematic review. *Am J Med* 2003; 115:554-9.

25. Corriveau P, Paquette A, Brochu M, Prud'homme D, Rabasa-Lhoret R, Lavoie JM. Resistance training prevents liver fat accumulation in ovariectomized rats. *Maturitas* 2008; 59:259-67.

26. Rainville S, Vaccaro P. The effects of menopause and training on serum lipids. *Int J Sports Med* 1984; 5:137-41.

27. Robertson MC, Owens RE, Klindt J, Friesen HG. Ovariectomy leads to a rapid increase in rat placental lactogen secretion. *Endocrinology* 1984; 114:1805-11.

28. El-Mas MM, Abdel-Rahman AA. Effects of long-term ovariectomy and estrogen replacement on clonidine-evoked reductions in blood pressure and hemodynamic variability. *J Cardiovasc Pharmacol* 2004; 43:607-15.

29. Krotkiewski M, Bjorntorp P. The effect of progesterone and of insulin administration on regional adipose tissue cellularity in the rat. *Acta Physiol Scand* 1976; 96:122-7.

30. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; 28:412-9.

31. McElroy JF, Wade GN. Short- and long-term effects of ovariectomy on food intake, body weight, carcass composition, and brown adipose tissue in rats. *Physiol Behav* 1987; 39:361-5.

32. Richard D. Effects of ovarian hormones on energy balance and brown adipose tissue thermogenesis. *Am J Physiol* 1986; 250:R245-9.

33. Mukherjea M, Biswas R. Effect of ovariectomy and replacement therapy on the tissue lipid pattern in rats. *Endokrinologie* 1975; 66:113-21.

34. Barsalani R, Pighon A, Rabasa-Lhoret R, Yasari S, Lavoie JM. Liver of ovariectomized rats is resistant to resorption of lipids. *Physiol Behav* 2008; 95:216-21.

35. Paquette A, Wang D, Jankowski M, Gutkowska J, Lavoie JM. Effects of ovariectomy on PPAR alpha, SREBP-1c, and SCD-1 gene expression in the rat liver. *Menopause* 2008; 15:1169-75.

36. Rachon D, Vortherms T, Seidlova-Wuttke D, Wuttke W. Effects of black cohosh extract on body weight gain, intra-abdominal fat accumulation, plasma lipids and glucose tolerance in ovariectomized Sprague-Dawley rats. *Maturitas* 2008; 60:209-15.

37. Levin BE, Dunn-Meynell AA. Chronic exercise lowers the defended body weight gain and adiposity in diet-induced obese rats. *Am J Physiol Regul Integr Comp Physiol* 2004; 286:R771-8.

38. Yasari S, Dufresne E, Prud'homme D, Lavoie JM. Effect of the detraining status on high-fat diet induced fat accumulation in the adipose tissue and liver in female rats. *Physiol Behav* 2007; 91:281-9.

39. Applegate EA, Upton DE, Stern JS. Exercise and detraining: effect on food intake, adiposity and lipogenesis in Osborne-Mendel rats made obese by a high fat diet. *J Nutr* 1984; 114:447-59.

40. Craig BW, Thompson K, Holloszy JO. Effects of stopping training on size and response to insulin of fat cells in female rats. *J Appl Physiol* 1983; 54:571-5.

41. Hill JO, Davis JR, Tagliaferro AR. Effects of diet and exercise training on thermogenesis in adult female rats. *Physiol Behav* 1983; 31:133-5.

42. Lambert EV, Wooding G, Lambert MI, Koeslag JH, Noakes TD. Enhanced adipose tissue lipoprotein lipase activity in detrained rats: independent of changes in food intake. *J Appl Physiol* 1994; 77:2564-71.

43. Paquette A, Chapados NA, Bergeron R, Lavoie J-M. Fatty acid oxidation is reduced in liver of ovariectomized rats. *Horm Met Res* 2009; 41:1-5. (**DOI** 10.1055/s-0029-1202348).

Table 1. Uterus weight, sum of weights of four leg muscles and plasma glucose, insulin, triacyglycerol (TAG), free fatty acid (FFA) concentrations in sedentary (Sed) and training (Tr) rats for 6 weeks before surgery and subsequently divided into groups of sham-operated (Sham) and ovariectomized rats without (Ovx), and with 17β-estradiol replacement (OvxE2) evaluated 6 weeks after surgery (total 12 weeks). Pre-surgery sedentary rats either remained sedentary (Sed-Sed) or started training (Sed-Tr) after surgery while pre-surgery trained rats either stopped (Tr-Sed) or kept on training (Tr-Tr) after surgery. Values are mean ± standard errors; n = 8 rats/group

Time		*Group*					
(weeks)	*Surgery*	*Sed*	*Tr*	*Sed-Sed*	*Sed-Tr*	*Tr-Sed*	*Tr-Tr*
Uterus weight (mg)							
6		558 ± 51	558 ± 44				
12	*Sham*			602 ± 51	629 ± 50	588 ± 45	632 ± 44
12	*Ovx*			123 ± 9^{b}	123 ± 8^{b}	137 ± 9^{b}	136 ± 8^{b}
12	*OvxE2*			774 ± 64^{c}	798 ± 41^{c}	766 ± 62^{c}	781 ± 55^{c}
Leg muscles weight (g)							
6		2.1 ± 0.07	2.4 ± 0.04^{a}				
12	*Sham*			2.1 ± 0.08	2.3 ± 0.07	2.3 ± 0.07	2.3 ± 0.09
12	*Ovx*			2.3 ± 0.08^{b}	2.5 ± 0.09^{b}	2.5 ± 0.05^{b}	2.4 ± 0.08^{b}
12	*OvxE2*			2.1 ± 0.05	2.1 ± 0.05	2.1 ± 0.06	2.1 ± 0.05
Plasma glucose (mmol/l)							
6		9.45 ± 0.2	9.23 ± 0.4				
12	*Sham*			8.62 ± 0.3	8.91 ± 0.2	9.17 ± 0.5	8.68 ± 0.3
12	*Ovx*			10.5 ± 0.4^{b}	9.59 ± 0.3^{b}	9.81 ± 0.5^{b}	9.34 ± 0.3^{b}
12	*OvxE2*			6.83 ± 0.3^{c}	7.28 ± 0.4^{c}	7.31 ± 0.3^{c}	6.98 ± 0.2^{c}
Plasma insulin (pmol/l)							
6		215 ± 33	153 ± 34				
12	*Sham*			263 ± 22	251 ± 28^{d,ee}	302 ± 31	202 ± 24^{d,ee}
12	*Ovx*			387 ± 52^{b}	250 ± 50^{b,d,ee}	346 ± 40^{b}	269 ± 37^{b,d,ee}
12	*OvxE2*			231 ± 34	161 ± 23^{d,ee}	278 ± 42	191 ± 23^{d,ee}
Plasma TAG (mmol/l)							
6		0.61 ± 0.1	0.45 ± 0.08				
12	*Sham*			0.82 ± 0.15	0.48 ± 0.06^{dd}	0.56 ± 0.06^{dd}	0.57 ± 0.08^{dd}
12	*Ovx*			0.72 ± 0.06	0.38 ± 0.08^{dd}	0.51 ± 0.08^{dd}	0.45 ± 0.04^{dd}
12	*OvxE2*			0.71 ± 0.09	0.61 ± 0.06^{dd}	0.6 ± 0.09^{dd}	0.52 ± 0.06^{dd}
Plasma FFA (mmol/l)							
6		0.14 ± 0.01	0.07 ± 0.02^{aa}				
12	*Sham*			0.13 ± 0.02	0.09 ± 0.02^{dd,e}	0.11 ± 0.02	0.1 ± 0.02
12	*Ovx*			0.09 ± 0.02^{b}	0.06 ± 0.01^{b,dd,e}	0.1 ± 0.02^{b}	0.08 ± 0.01^{b}
12	*OvxE2*			0.13 ± 0.02	0.09 ± 0.01^{dd,e}	0.12 ± 0.01	0.11 ± 0.02

[a], Significantly different from Tr group, $P < 0.05$, [aa], $P < 0.01$; [b], surgery effect: significantly different from Sham and OvxE2 groups, $P < 0.05$; [c], surgery effect: significantly different from Sham group, $P < 0.05$; [d], treatment effect: significantly different from Sed-Sed groups, $P < 0.05$, [dd], $P < 0.01$; [e], treatment effect: significantly different from Tr-Sed groups, $P < 0.05$, [ee], $P < 0.01$

LEGENDS

Figure 1. Schematic representation of the experimental design. The tip of the arrow indicates the time of sacrifice. Sham, sham-operated; Ovx, ovariectomized; OvxE2, ovariectomized þ 17β-estradiol replacement. Pre-surgery sedentary (Sed) rats either remained sedentary (Sed-Sed) or started training (Sed-Tr) after surgery while pre-surgery training (Tr) rats either stopped (Tr-Sed) or kept on training (Tr-Tr) after surgery (n = 8 rats/group)

Figure 2. Final body weight (a) and daily energy intake (b) in sedentary (Sed) and training (Tr) rats for 6 weeks before surgery and subsequently divided into groups of sham-operated (Sham) and ovariectomized rats without (Ovx) and with 17 β-estradiol replacement (OvxE2) evaluated 6 weeks after surgery (total 12 weeks). Pre-surgery Sed rats either remained sedentary (Sed-Sed) or started training (Sed-Tr) after surgery while pre-surgery Tr rats either stopped (Tr-Sed) or kept on training (Tr-Tr) after surgery. Values are mean ± standard error, n = 8 rats/group. *, Surgery effect: significantly different from Sham and OvxE2 groups, $P < 0.01$; #, surgery effect: significantly different from Sham group, $P < 0.01$; ++, treatment effect: significantly different from Sed-Sed groups, $P < 0.01$; §§, treatment effect: significantly different from Tr-Sed groups, $P < 0.01$

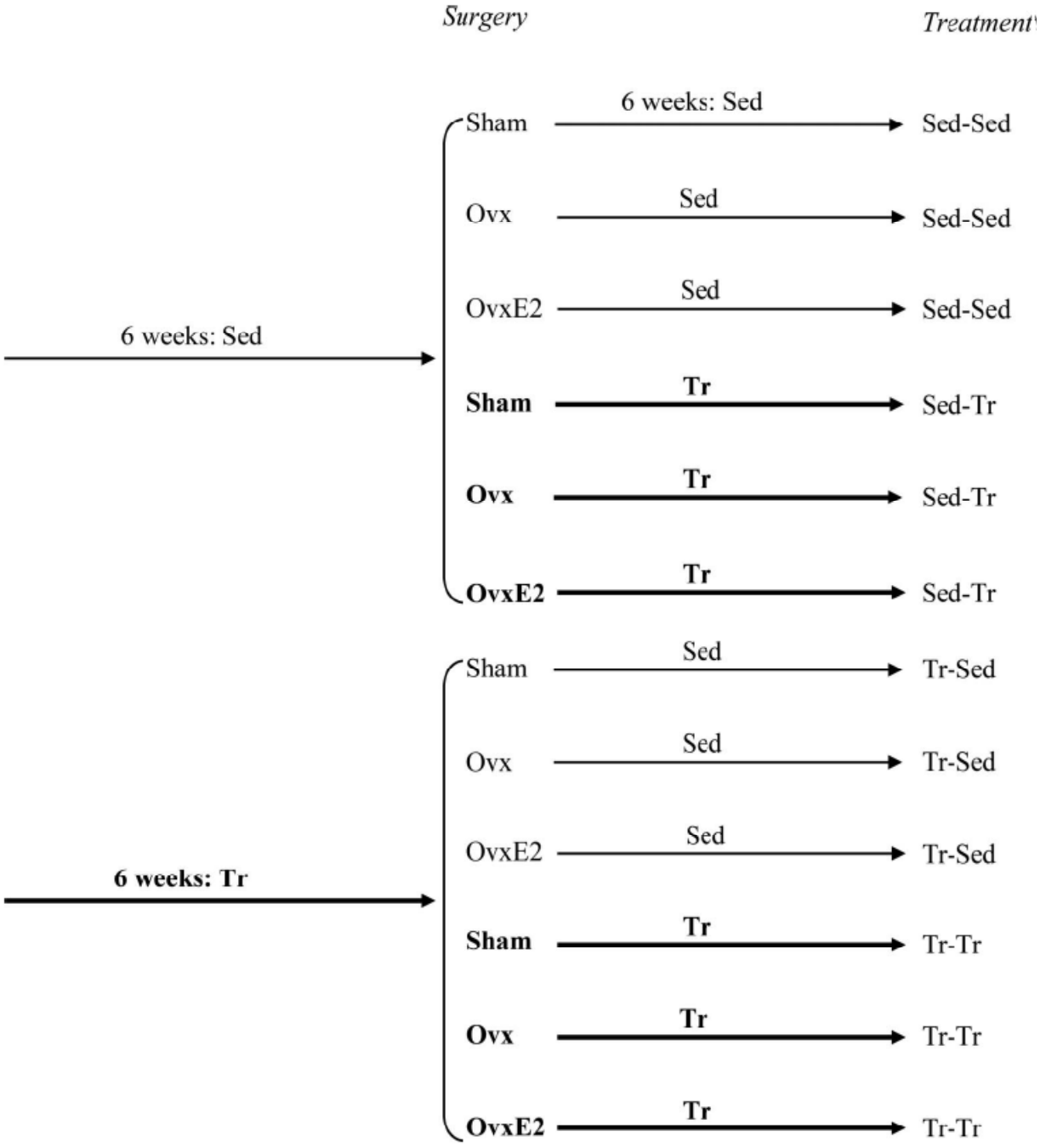
Figure 3. Sum of the weights of three intra-abdominal fat pads (a) and subcutaneous fat pad weight (b) in sedentary (Sed) and training (Tr) rats for 6 weeks before surgery and subsequently divided into groups of sham-operated (Sham) and ovariectomized rats without (Ovx) and with 17β-estradiol replacement (OvxE2) evaluated 6 weeks after surgery (total 12 weeks). Pre-surgery Sed rats either remained sedentary (Sed-Sed) or started training (Sed-Tr) after surgery while pre-surgery Tr rats either stopped (Tr-Sed) or kept on training (Tr-Tr) after surgery. Values are mean ± standard error, n = 8 rats/group. [a],

Significantly different from Tr group, $P < 0.05$; * surgery effect: significantly different from Sham and OvxE2 groups, $P < 0.01$; ++, treatment effect: significantly different from Sed-Sed groups, $P < 0.01$; §§; treatment effect: significantly different from Tr-Sed groups, $P < 0.01$

Figure 4. HOMA-IR values in sedentary (Sed) and training (Tr) rats for 6 weeks before surgery and subsequently divided into groups of sham-operated (Sham) and ovariectomized rats without (Ovx) and with 17β-estradiol replacement (OvxE2) evaluated 6 weeks after surgery (total 12 weeks). Pre-surgery Sed rats either remained sedentary (Sed-Sed) or started training (Sed-Tr) after surgery while pre-surgery Tr rats either stopped (Tr-Sed) or kept on training (Tr-Tr) after surgery. Values are mean ± standard error, $n = 8$ rats/group. *, Surgery effect: significantly different from Sham and OvxE2 groups, $P < 0.01$; #, surgery effect: significantly different from Sham group, $P < 0.01$; +, treatment effect: significantly different from Sed-Sed groups, $P < 0.05$, ++ $P < 0.01$; §§, treatment effect: significantly different from Tr-Sed groups, $P < 0.01$. HOMA-IR, homeostatic model assessment of insulin resistance [glucose (mmol/l) x insulin (mIU/l)/22.5], (3-h fasted state)

Figure 5. Liver triacylglycerol (TAG) concentrations in sedentary (Sed) and training (Tr) rats for 6 weeks before surgery and subsequently divided into groups of sham-operated (Sham) and ovariectomized rats without (Ovx) and with 17β-estradiol replacement (OvxE2) evaluated 6 weeks after surgery (total 12 weeks). Pre-surgery Sed rats either remained sedentary (Sed-Sed) or started training (Sed-Tr) after surgery while pre-surgery Tr rats either stopped (Tr-Sed) or kept on training (Tr-Tr) after surgery. Values are mean ± standard error, $n = 8$ rats/group. *, Surgery effect; significantly different from Sham and OvxE2 groups, $P < 0.01$; +, treatment effect: significantly different from Sed-Sed groups, $P < 0.05$; §, treatment effect: significantly different from Tr-Sed groups, $P < 0.05$

Figure 1.


Surgery
Treatment
6 weeks: Sed
6 weeks: Tr
Sham
Ovx
OvxE2
Sham
Ovx
OvxE2
Sham
Ovx
OvxE2
6 weeks: Sed
Sed
Sed
Tr
Tr
Tr
Sed
Sed
Sed
Tr
Tr
Tr
Sed-Sed
Sed-Sed
Sed-Sed
Sed-Tr
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Tr-Sed
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Tr-Tr
Tr-Tr
Tr-Tr

Figure 2.

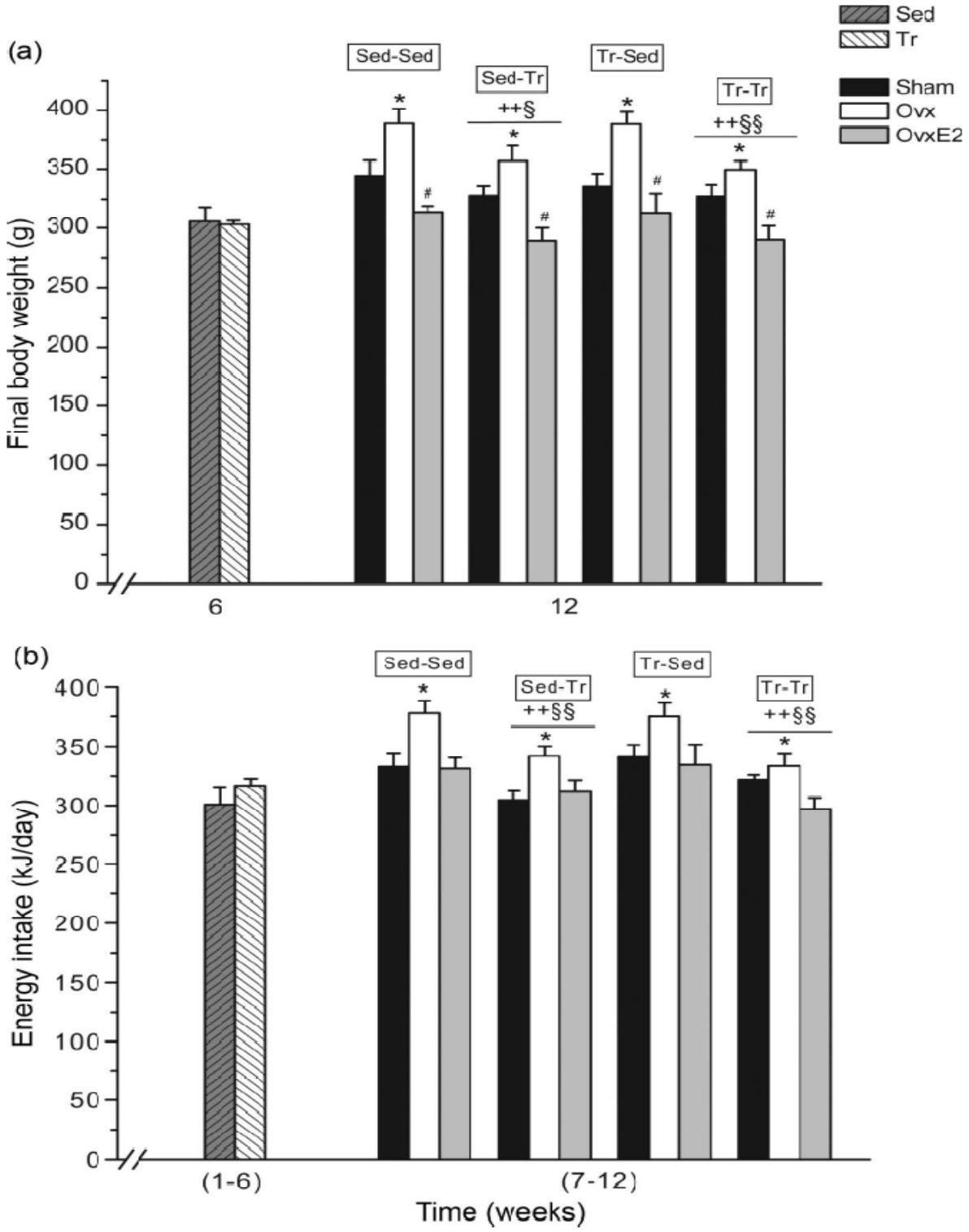

(a)
Sed
Tr
Sham
Ovx
OvxE2
Sed-Sed
Sed-Tr
++§
Tr-Sed
Tr-Tr
++§§
Final body weight (g)
0
50
100
150
200
250
300
350
400
6
12
(b)
Sed-Sed
Sed-Tr
++§§
Tr-Sed
Tr-Tr
++§§
Energy intake (kJ/day)
0
50
100
150
200
250
300
350
400
(1-6)
(7-12)
Time (weeks)

Figure 3.

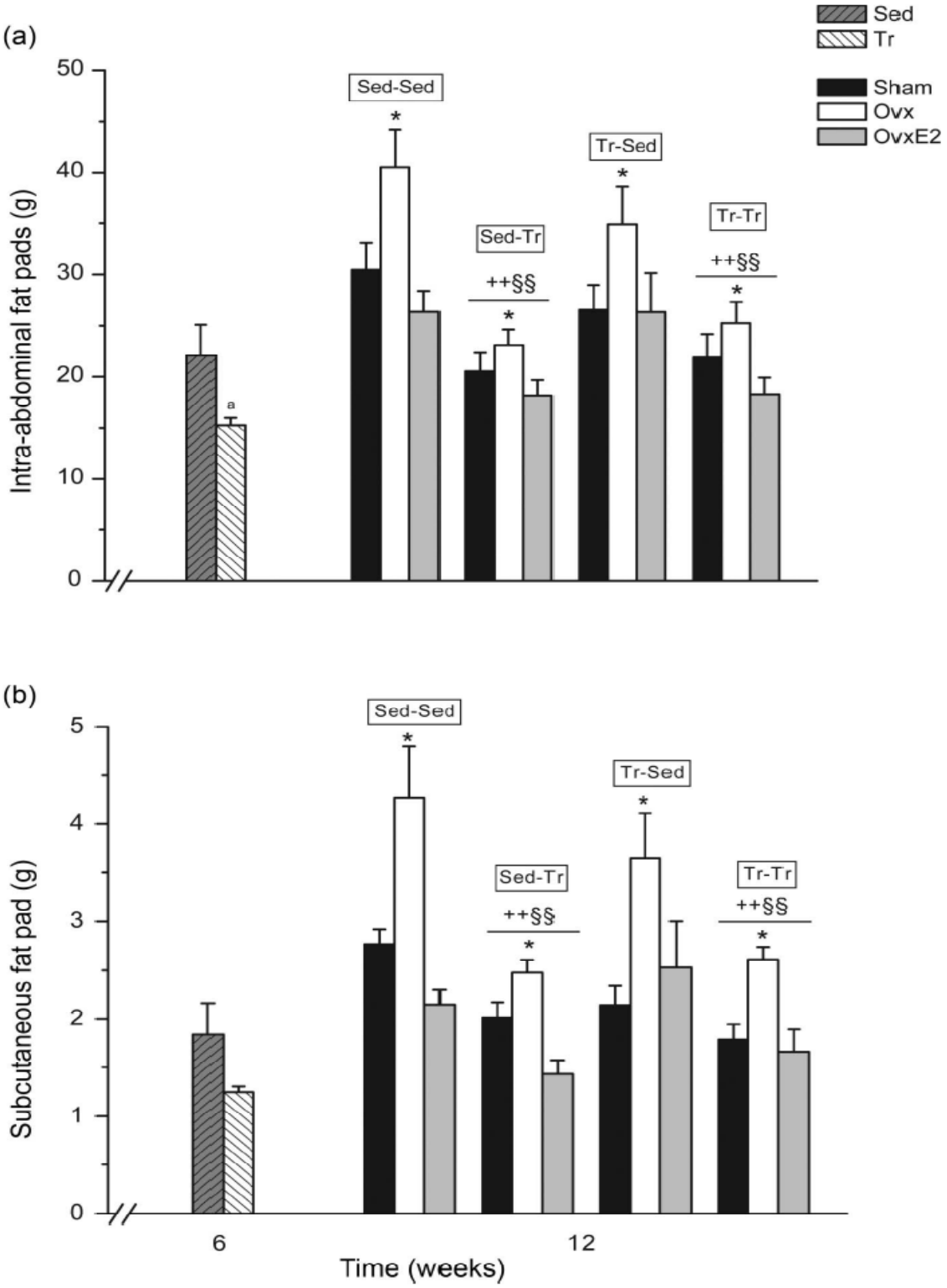

(a)
Sed
Tr
Sham
Ovx
OvxE2
Intra-abdominal fat pads (g)
50
40
30
20
10
0
Sed-Sed
Sed-Tr
Tr-Sed
Tr-Tr
++§§
*
a
(b)
Subcutaneous fat pad (g)
5
4
3
2
1
0
6
12
Time (weeks)

Figure 4.

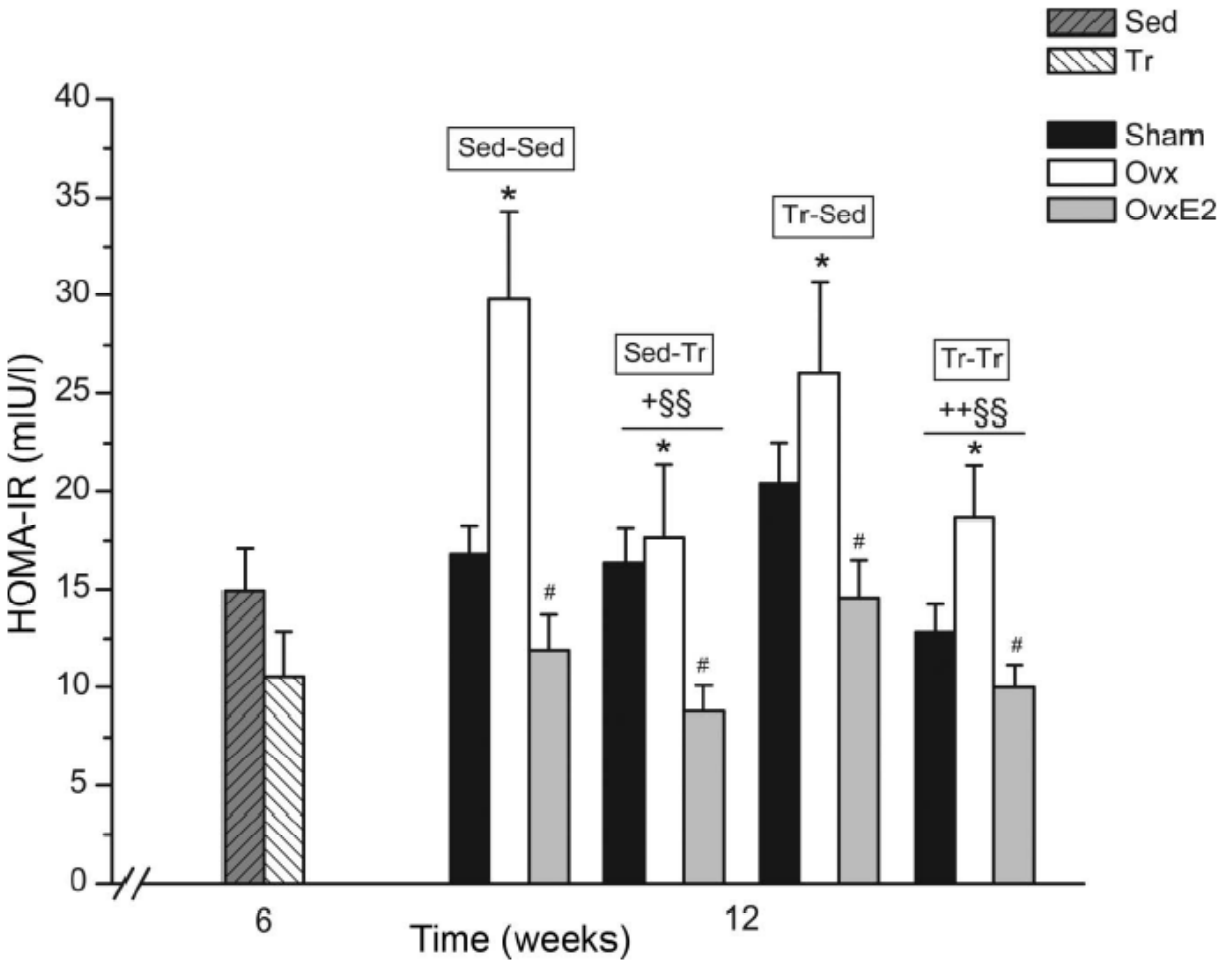
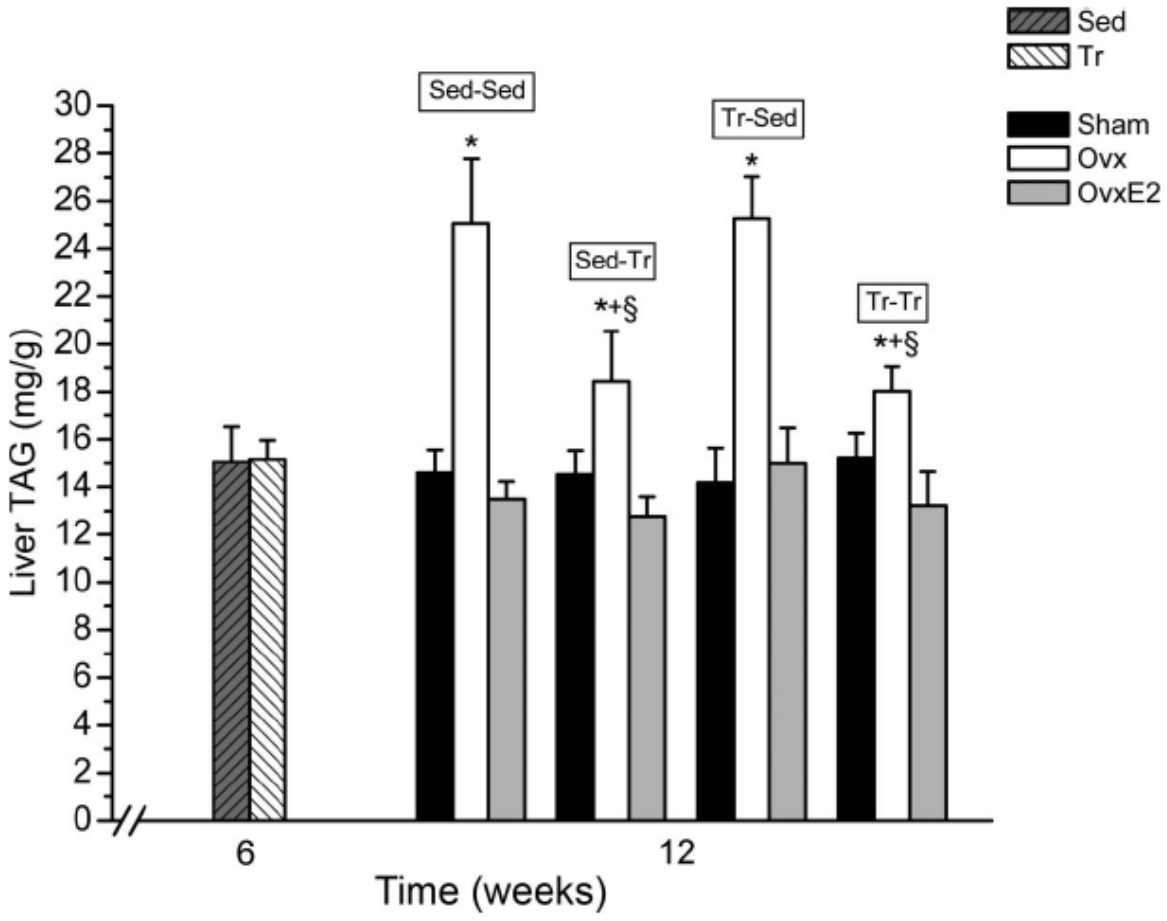

Sed
Tr
Sham
Ovx
OvxE2
HOMA-IR (mIU/l)
40
35
30
25
20
15
10
5
0
Sed-Sed
Sed-Tr
Tr-Sed
Tr-Tr
+§§
++§§
*
#
6
12
Time (weeks)

Figure 5.


Sed
Tr
Sham
Ovx
OvxE2
Sed-Sed
Sed-Tr
Tr-Sed
Tr-Tr
*
*+§
*
*+§
Liver TAG (mg/g)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
6
12
Time (weeks)

Chapter 5: Original article 4

Title:

Exercise training in ovariectomized rats stimulates estrogenic-like effects on the expression of genes involved in lipid accumulation and sub-clinical inflammation in liver.

Authors:

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Exercise training in ovariectomized rats stimulates estrogenic-like effects on expression of genes involved in lipid accumulation and sub-clinical inflammation in liver.

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The experiments described in this report were conducted according to the directives of the Canadian Council on Animal Care after institutional approval.

Abstract

Objective: We hypothesized that the reduction in liver fat accumulation known to occur with exercise training in ovariectomized rats is associated with reduced expression of genes involved in lipogenesis while favoring the expression of transcription factors regulating lipid oxidation. We also tested the hypothesis that liver fat accumulation in Ovx rats is associated with an increased gene expression of several pro-inflammatory markers and that exercise training would attenuate this response.

Methods: Sprague-Dawley female rats (14 wk of age) were randomly divided into four groups of sedentary sham-operated (Sham), ovariectomized (Ovx), and Ovx with 17β-estradiol supplementation (OvxE2), and one group of endurance exercise trained Ovx (OvxTr). Endurance exercise training consisted of continuous running on a motor-driven rodent treadmill 5 times/wk for 5 wk.

Results: Fat accumulation in liver as well as in adipose fat depots was higher ($P < 0.01$) in Ovx than in Sham rats. This response was prevented in OvxE2 and OvxTr animals. Liver gene expressions of sterol regulatory element-binding protein 1-c (SREBP-1c), stearoyl coenzyme A desaturase 1 (SCD-1), carbohydrate response element binding protein (ChREBP), and acetyl-CoA carboxylase (ACC) were increased with estrogens withdrawal ($P < 0.01$). These responses were corrected with E2 supplementation alone as well as with training alone. Conversely, hepatic peroxisome proliferator-activated receptor α (PPAR-α) mRNA levels were lower ($P<0.01$) after estrogen removal compared to Sham rats. The lower hepatic PPAR-α mRNA levels in Ovx rats were re-increased by E2 replacement or by exercise training. Gene expression of pro-inflammatory cytokines including inhibitor-kappaB kinase β (IKKβ) and interleukin-6 (IL-6) as well as protein content of nuclear factor-kappa B (NF-κB) was higher ($P < 0.01$) in Ovx than in Sham animals. As for the metabolic markers, E2 supplementation or exercise training prevented the expression of the pro-inflammatory markers.

Conclusion: It is concluded that exercise training, similarly to estrogens reduces fat accumulation in liver of Ovx rats possibly through regulation of key molecules involved in lipogenesis and lipid oxidation. Exercise training also acts as estrogens in properly regulating the expression of inflammatory bio-markers in liver of Ovx rats.

Key words: Physical activity, Fatty liver, Estrogens, Lipogenesis, Lipid oxidation, hepatic inflammation, Menopause.

Introduction

Menopause is associated with the development of a state of hepatic steatosis (1, 2). The importance of this phenomenon is enlightened by the fact that excessive fat accumulation in liver plays an important role in the development of insulin resistance (3). Recent findings even indicate that ectopic fat in liver may be more important than visceral fat in characterization of metabolically benign obesity in humans (4, 5). Hepatic and adipocytes fat accumulation is also well documented in animal models of menopause (6-9). Ovariectomized (Ovx) rats as well as aromatase receptor knockout mice exhibit hepatic fat accumulation that seems to be triggered by changes in expression of genes that increase lipid synthesis and reduce lipid oxidation in liver (10, 11). There is also recent physiological evidence that fatty acid oxidation is reduced in liver of Ovx rats (12). An alternative to counteract liver fat accumulation with estrogen withdrawal might be exercise training. It was reported that exercise training prevents fat accumulation in liver of high-fat fed rats (13, 14). Specifically in Ovx rats, there is some evidence that resistance training in conjunction with food restriction reduces liver fat accumulation (15, 16). Recently, we reported that endurance exercise training when conducted concurrently with estrogen withdrawal prevented liver fat accumulation in Ovx rats (17). These studies, however, did not provide any mechanistic information on the action of exercise training in preventing liver fat accumulation in Ovx animals. The first aim of the present study was to test the hypothesis that exercise training reduces the expression of key molecules involved in lipid synthesis while favoring the expression of molecules involved in fat oxidation.

In recent years, it has become clear that metabolic disturbances related to fat accumulation in adipocytes and ectopic tissues, such as liver, are associated with sub-clinical inflammation (18, 19). For instance, Cai *et al.* showed that inflammatory gene expression increases in liver of both transgenic and high-fat fed mice with increasing adiposity (20). Regardless of the causes, the nuclear factor-kappa B (NF-κB) is activated in hepatocytes and pro-inflammatory cytokines including tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6) are overproduced in fatty liver (21). As such, it is relevant to

investigate if liver lipid accumulation resulting from estrogens deficit in Ovx rats leads to sub-acute hepatic inflammation and if it is so, whether exercise training attenuates this response as it does for fat accumulation. Consequently, the second objective of the present study was to investigate the effects of ovariectomy and exercise training on gene expression of inflammatory bio-markers in the liver.

Methods

Animal care. Female Sprague-Dawley strain rats (Charles River, St-Constant, PQ, Canada), weighing 180-200g upon their arrival were housed individually and had *ad libitum* access to food and tap water. All rats received usual pellet rat chow, referred to as the standard diet (SD; 12.5% fat; 63.2% carbohydrate; 24.3% protein; kcal, Agribrands Purina Canada, Woodstock, ON). 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). The experiments described in this report were conducted according to the directives of the Canadian Council on Animal Care after institutional approval.

Groups. 6 weeks after their arrival to our laboratory, rats were randomly divided into sedentary sham-operated (Sham), sedentary ovariectomized (Ovx), sedentary Ovx with 17β-estradiol supplementation (OvxE2), and Ovx rats that underwent endurance exercise training (OvxTr) groups (n = 6-8 rats / group). Body weight and food intake were monitored three times per week. All animals were sacrificed 6 weeks after the surgical manipulations.

Surgery. Ovariectomy surgery was conducted according to the technique described by Robertson *et al*. (22). Animals were injected with antibiotics (Tribrissen 24%; 0.125cc/kg, SC) for three days, beginning the day before surgery. For surgery, rats were anesthetized with a mixture of ketamine-xylazine (61.5-7.6 mg/kg, IP). In OvxE2 rats, a small 17β-estradiol pellet (0.72 mg; 0.012 mg/d) with a biodegradable carrier binder efficient for 60 days (catalog no. SE-121; Innovative Research of America, Sarasota, FL) was placed SC between the shoulder blades. El-Mas and Abdel-Rahman previously showed that this estrogen regimen produces physiological levels of the hormone (23). A placebo 60-day pellet containing the binding carrier only was used in all other rats (catalog no. SC-111).

Exercise protocol. One week (7^{th} wk) was provided for surgery and recovery in all rats. During this time, all animals were submitted to a habituation running protocol (10-15

min/day) in the last 3 days of the week. Exercise training (Tr) consisted of continuous running on a motor-driven rodent treadmill (Quinton Instruments, Seattle, WA), 5 times per week for the duration of the experiment (5 weeks after recovery). OvxTr rats progressively ran from 15 min/day at 15 m/min, 0% slope, up to 60 min/day at 26 m/min, 10% slope for the last 3 wk. All Tr animals were restrained from training 48 h before sacrifice.

Blood and tissue sampling. Rats were sacrificed between 09:00 and 12:00 AM. Food was removed from the animal's cage at least 3 h before sacrifice. Immediately after complete anesthesia (pentobarbital sodium; 50mg/kg, IP), the abdominal cavity was opened following the median line of the abdomen and approximately 4 mL of blood was collected from the abdominal vena cava (<45s) into syringes pre-treated with ethylenediaminetetraacetic acid (15%; EDTA). Blood was centrifuged (3000 rpm; 4°C; 12 min; Beckman GPR Centrifuge) and the plasma was kept for further analysis. The liver was excised and the median lobe was immediately snap-frozen and was used for triacylglycerol (TAG) determination, mRNA extraction and quantification, and Western blotting. The uterus, mesenteric and retroperitoneal fat pads along with 4 skeletal muscles of the right hind limb (soleus, plantaris, medial gastrocnemius, and lateral gastrocnemius) were, thereafter, rapidly excised and weighed. All tissue and plasma samples were stored at -78° C until analyses were performed. Finally, the right femur wet weight was obtained following a short boiling period in a 10% KOH solution in order to remove the surrounding tissue.

Biochemical analyses. Plasma insulin and leptin concentrations were determined with radioimmunoassay test kit distributed by LINCO Research (St. Charles, Missouri, USA). Plasma glucose concentrations were determined with the use of a glucose analyzer (Yellow Springs Instruments 2300, Yellow Springs, OH). Plasma glucose and insulin values were used to calculate a homeostasis model assessment of insulin resistance (HOMA-IR) as follows: glucose (mmol/l) × insulin (mIU/L)/22.5 (24). Plasma CRP concentrations were measured with Synchron LX Systems (Beckman Coulter) using Alpco Diagnostics kit (cat. no 41-CRPRT-E01). Liver TAG concentrations were estimated from

glycerol released after ethanolic KOH hydrolysis by using commercial kit from SIGMA (Saint Louis, Missouri, USA).

Isolation of RNA and quantitative real-time (RT) polymerase chain reaction (PCR): *RNA extraction and cDNA preparation.* Quick-frozen tissue samples of the liver were powdered with cold mortar and pestle, and approximately 100 mg was used for the isolation of RNA. Total RNA was extracted by the guanidine thiocyanate method and mRNA purified using PureLink RNA Mini Kit (Invitrogen) according to the manufacturer's instruction. Total RNA were reverse transcribed in a final volume of 100 µL using the High Capacity cDNA Reverse Transcription Kit with random primers (Applied Biosystems, Foster City, CA) as described by the manufacturer. Reverse transcribed samples were stored at -20°C. A reference RNA (Human reference total RNA, Stratagene, Ca) was also transcribed in cDNA.

qPCR Reactions- ABI Gene Expression Assay – Endogenous controls. Gene expression level was determined using primer and probe sets from Applied Biosystems (ABI Gene Expression Assays, http://www.appliedbiosystems.com). PCR reactions for 384 well plate formats were performed using 2 µl of cDNA samples (20-50 ng), 5 µl of the Express qPCR SuperMix (Invitrogen), 0.5 µl of the TaqMan® Gene Expression Assays (20X) and 2.5 µl of water in a total volume of 10 µl. The following pre-developed TaqMan® assays were used as endogenous control: GAPDH (glyceraldehyde-3-phosphate dehydrogenase).

TaqMan reactions using Universal Probe Library. Gene expression level was also determined using primer and probe sets from Universal ProbeLibrary from Roche, a fast, specific and flexible format for quantitative real-time PCR (https://www.roche-applied-science.com/sis/rtpcr/upl/index.jsp). PCR reactions for 384 well plate formats were performed using 2 µl of cDNA samples (50 ng), 5 µl of the Express qPCR SuperMix (Invitrogen), 2 µM of each primer and 1 µM of the Universal TaqMan probe in a total volume of 10 µl. The primer sets served to generate amplicons are presented in Table 1.

Detection and analysis. The ABI PRISM® 7900HT Sequence Detection System (Applied Biosystems) was used to detect the amplification level and was programmed FAST with an initial step of 3 min at 95˚C, followed by 45 cycles of 5 seconds at 95˚C and 30 seconds at 60˚C. All reactions were run in triplicate and the average values were used for quantification. GAPDH (glyceraldehyde-3-phosphate dehydrogenase) was used as endogenous control. The relative quantification of target genes was determined using the △△CT method. Briefly, the Ct (threshold cycle) values of target genes were normalized to an endogenous control gene (GAPDH) ($\triangle CT = Ct_{target} - Ct_{GAPDH}$) and compared with a calibrator: $\triangle\triangle CT = \triangle Ct_{Sample} - \triangle Ct_{Calibrator}$. Relative expression (RQ) was calculated using the Sequence Detection System (SDS) 2.2.2 software (Applied Biosystems): $RQ = 2^{-\triangle\triangle CT}$.

Western blot analysis. 100 mg of liver was homogenized in TPER containing protease inhibitors (10 μl/ml pepstatin, and 1 mM phenylmethanesulfonyl fluoride and 100U Trasylol) using a polytron and centrifuged at 12000g, 4°C for 10 min. The infranatant was collected with a blunt-tipped Pasteur pipette and stored at −80°C until protein determination. SCD-1 and NFκB contents in the liver were determined by Western blotting. All samples (10μg of proteins) were separated on a SDS-polyacrylamide gel and electro-transferred onto Hybond-Cextra nitrocellulose membranes (Amersham). Membranes were blocked overnight in Tris-buffered saline containing 0.05% Tween 20 (TBST 0.05%) and 5% nonfat dry milk at 4°C. The blot was then incubated with specific primary antibodies: SCD-1 (kindly provided by Dr. J. Ozols, University of Connecticut Health Center, Storrs, CT) and NFκB p65 (sc-109, Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4°C. After two washes in TBST (0.05%) and two washes in TBST (0.05%) containing 0.5% nonfat dry milk, the membrane was incubated for 30 min with a horseradish peroxidase-conjugated anti-rabbit/anti-mouse IgG (Bm chemiluminescence Western Blotting Kit, cat. no. 11520709001, Roche Diagnostics) at room temperature. Then the membrane was washed four times for 20 min each time in TBST (0.05%) before a chemiluminescence substrate (cat. no. 11520709001, Roche Diagnostics) was applied to the membrane. The resulting signal was detected on scientific imaging films (Amersham). Densitometric

measurement of the bands was performed using Image J software and expressed as arbitrary units. Equal protein loading was determined using mouse anti-β-actin primary antibodies (Sigma, Saint Louis, Missouri, USA).

Statistical analysis. Values are expressed as mean ± S.E. Statistical analyses were performed using one-way ANOVA for non-repeated measures. Fisher's PLSD *post-hoc* test was used in the event of a significant ($P < 0.05$) F ratio.

Results

Ovariectomy in rats, as compared to Sham operation resulted in higher body weight ($P<0.05$), daily energy intake, mesenteric and retroperitoneal fat depots weights ($P<0.01$) as well as higher plasma concentrations of insulin ($P=0.059$), glucose, leptin, and HOMA-IR index ($P < 0.01$; Table 2). Furthermore, Ovx resulted in lower femur (14%; $P<0.05$) and uterus weight (80%; $P<0.01$) indicating physiological effects of ovariectomy. As a rule, almost all changes induced by ovariectomy were prevented either by 17β-estradiol supplementation or endurance exercise training (Table 2). The exceptions were uterus weight and an increase in plasma glucose concentrations in OvxTr rats. On the other hand, the higher uterus weight (29%; $P<0.01$) in OvxE2 compared with Sham rats suggests that 17β-estradiol supplementation was slightly supraphysiological. There was no significant difference between Sham and Ovx rats in relative muscle weight (Table 2). However, exercise training and 17β-estradiol supplementation in Ovx rats increased leg muscles weight ($P<0.01$).

Liver TAG levels were 71% higher (25.1±2.7 vs 14.6±1 mg/g; $P<0.01$) in Ovx than in Sham rats (Fig. 1A). The ovx-induced hepatic fat accumulation was prevented either by 17 β-estradiol replacement or exercise training. Quantitative real-time polymerase chain reaction (RT-PCR) analysis showed higher ($P<0.01$) gene expression of hepatic lipogenic transcription factors sterol regulatory element-binding protein 1-c (SREBP-1c) (57%; Fig. 1B), stearoyl coenzyme A desaturase 1 (SCD-1) (87%; Fig. 2A), and carbohydrate response element binding protein (ChREBP) (63%; Fig. 2C), as well as acetyl-CoA carboxylase (ACC) (68%; Fig. 2D) mRNA levels in Ovx than in Sham rats. Protein quantification by Western blot analysis confirmed results obtained by RT-PCR for SCD-1. We found greater SCD-1 (40%; $P<0.01$; Fig. 2B) protein abundance in the liver of Ovx rats. These Ovx-induced higher lipogenic gene expressions and protein content were totally prevented either by E2 replacement or by endurance training. Conversely, the hepatic oxidative transcription factor peroxisome proliferator-activated receptor α (PPAR-α) mRNA was lower (31%; $P<0.01$) in Ovx than in Sham rats while hepatic peroxisome proliferator-activated receptor-

γ coactivator 1α (PGC-1α) mRNA showed the same trend, although the differences did not reach significant statistical levels (Fig. 3). Again, lower PPAR-α mRNA levels were re-established in OvxE2 animals and Ovx trained rats.

To get an insight into how estrogen withdrawal and endurance exercise training affect the hepatic inflammatory response, we measured gene expression of several inflammatory markers in liver. Ovx, as compared to Sham rats, resulted in higher (P<0.01) hepatic pro-inflammatory IL-6 (37%) and inhibitor of kappa B kinase beta (IKKB) (35%) mRNA expressions (Fig. 4). Moreover, we found higher NF-κB (51%; P<0.01; Fig. 4C) protein level in liver of Ovx rats than in Sham control. Likewise the metabolic markers, Ovx-induced changes of inflammatory markers were totally prevented with estrogen replacement as well as with endurance training. TNF-α and interleukin 10 (IL-10) mRNA were not significantly changed by the ovariectomy (Fig. 5). Nevertheless, TNF-α gene expression was significantly (P<0.05) lower in OvxE2 and OvxTr rats compared to Ovx animals. Interestingly, the ratio IL-10/TNF-α was higher (P<0.05) in OvxE2 and OvxTr animals compared to Ovx group indicating better inflammatory status under these physiological conditions (E2 supplementation and exercise training) (Fig. 5).

Opposite to the other inflammatory markers, we found that plasma C reactive protein (CRP) level as well as hepatic CRP mRNA expression were significantly lower (P<0.01) in Ovx rats than in Sham animals (Fig. 6). These responses were re-increased when E2 replacement was provided. On the other hand plasma CRP remained decreased in OvxTr and hepatic CRP mRNA level in this group was midway between Ovx and OvxE2 groups at the Sham group's level.

Discussion

The present observation that liver TAG content was 71% higher in Ovx than in Sham rats is consistent with previous findings showing that estrogen withdrawal results in a state of hepatic steatosis (7, 9, 25). We also confirmed previous reports that 17β-estradiol supplementation as well as endurance exercise training prevents the accretion of lipids in the liver of Ovx rats (10, 12, 17). This indicates that endurance exercise training has an important estrogenic-like effect on the prevention of hepatic steatosis in Ovx animals. It is important to recall that pair-feeding in Ovx rats does not completely prevent fat accumulation in liver ((26) and unpublished data from our lab). Consequently, Ovx-induced hepatic fat accumulation cannot be totally attributed to the increased food intake. To shed some light on possible mechanisms involved in the metabolic action of exercise training in liver of Ovx rats, we analyzed the expression of influential genes that regulate hepatic fat accumulation.

Kinetic studies in human subjects indicate that ~26% of hepatic TAG accumulation can be accounted for by *de novo* lipogenesis (27). Therefore, it has been assumed that the enhancement of *de novo* lipid synthesis is a primary disorder in hepatic steatosis which is tightly stimulated by lipogenic molecules such as the transcription factor SREBP-1c and the ACC downstream enzyme (28). Similarly, SCD-1 is an enzyme that represents a pivotal control point in lipid homeostasis by catalyzing a rate-limiting step in the biosynthesis of monounsaturated fats, which are required for TAG synthesis (29, 30). On the other hand, the transcription factor ChREBP also plays an essential role in the regulation of gene expression of enzymes (i.e. ACC and fatty acid synthase (FAS)) involved in lipogenesis derived from glucose metabolism (31). Recently, it was reported that ovariectomy increased SREBP-1c and SCD-1 gene expressions (10). The present results complement these previous findings by showing that the gene expression of transcription factor ChREBP and the important downstream ACC enzyme are also elevated in Ovx rats. The main contribution of the present study, however, is the original finding that endurance exercise training, similarly to E2 supplementation, counteracted these hepatic molecular

disturbances by cancelling the increase in SREBP-1c, SCD-1, ChREBP, and ACC transcripts in Ovx rats. This effect was confirmed at the protein level for SCD-1. These molecular responses strongly suggest that endurance training depressed lipogenesis in liver of Ovx rats, thus constituting a possible mechanism that contributes to the decrease in liver fat accumulation.

In addition to increased lipogenesis, recent data indicate that fatty acid oxidation is reduced in the liver of estrogen-deficient animals (10-12). The present decrease in gene expression of PPAR-α in liver of Ovx rats, which is re-increased in OvxE2 animals, is in agreement with this finding. PPAR-α is the key transcriptional regulator of peroxisomal, mitochondrial, and microsomal fatty acid oxidation systems in the liver (32, 33). Although less pronounced, PPAR-α transcript was also higher in OvxTr than in Ovx rats. This suggests that in addition to suppression of higher rates of liver lipogenesis, a re-increase in lipid oxidation in exercise trained Ovx rats, may also contribute to the prevention of liver lipid accumulation in Ovx rats.

The fact that exercise training acts similarly as estrogen supplementation in changing gene expression of key molecules involved in fat metabolism in the liver of Ovx rats raises the question if both of these actions take place through a similar pathway. The molecular and biological mechanisms underlying the metabolic actions of estrogen in liver are weakly understood. Estrogen (E2) is a steroid hormone whose actions are predominantly mediated by genomic mechanisms of E2 action through its nuclear receptors (ER) α or β (34). E2 has also been shown to have rapid non-genomic biological actions through membrane bound subpopulations of ER (35-37). D`Eon *et al.* recently reported that E2 treatment decreases gene expression of SREBP-1c and its target genes FAS and ACC in liver (38). It is possible that E2 directly regulates SREBP-1c which contains an estrogen response element (ERE) in its promoter region (39). On the other hand, D`Eon *et al.* showed that E2 rapidly activates AMP-activated protein kinase (AMPK) in skeletal muscle (38). Since SREBP-1c expression is down-regulated by AMPK (40), they suggested that the decreased expression of SREBP-1c in muscle reflects AMPK activation by non-

genomic action of E2 (38). Because physical exercise has been reported to activate AMPK in liver (41), it is thus possible that the decreased expression of SREBP-1c and its downstream targets (SCD-1 and ACC) by exercise training may be mediated through the AMPK pathway. Important increases in hepatic ERα mRNA levels have also been found in endurance-trained rats (42). Although further work is needed to clarify the precise mechanism, the present data support the contention that both E2 and exercise training act on hepatic expression of the same target genes to reduce *de novo* lipogenesis while favoring fat oxidation.

An additional support to the interpretation that exercise training acts similarly as estrogen supplementation is the finding that exercise training had similar effects as E2 supplementation in reducing peripheral fat accumulation and plasma leptin levels, as well as peripheral insulin resistance (as measured from HOMA-IR index) in the present Ovx rats. These results are in line with the recent report of Saengsirisuwan *et al.* who showed Ovx-induced features of the insulin resistance syndrome are largely attenuated by endurance exercise training alone and estrogen replacement alone (43). On the whole, the present results suggest that exercise training in Ovx rats acts similarly as estrogen supplementation in regulating not only liver fat but also peripheral fat accumulation and its metabolic consequence, the insulin resistance.

The second objective of the present study was to investigate if gene expressions of some important inflammatory biomarkers were increased in liver of Ovx rats and the impact of exercise training on this response. Regardless if the cause is the liver fat accumulation or the pro-inflammatory substances in the portal circulation; pro-inflammatory cytokines such as IL-6 and TNF-α are overproduced in fatty liver (21). Two main signaling pathways have been linked to inflammation associated with obesity: the NF-κB pathway, activated by IKKB; and the c-Jun NH2-terminal kinase (JNK) pathway (44). In recent years, it was found that the NF-κB and IKKB signaling pathway activated by pro-inflammatory cytokines is a key modulator of inflammation and insulin resistance (45). Cai *et al.* (20) demonstrated that lipid accumulation in liver (induced either by high-fat diet or

by transgenic expression of IKKB in mice) leads to sub-acute hepatic inflammation and downstream cytokine production, IL-6 showing the strongest evidence of pathological involvement. In the present study, mRNA levels of IL-6 and IKKB as well as protein content of NF-κB increased in liver of Ovx rats. E2 replacement neutralized these elevated gene expressions indicating that estrogens contribute to maintenance of low expression level of these inflammatory biomarkers in the liver. This is in line with results of Kireev *et al.* (46) and Hamilton *et al*. (47) who reported an increase in IL-6 and TNF-α gene expression in liver and heart of Ovx rats, respectively, that was corrected by 17β-estradiol replacement. To our knowledge, the present study is the first to report that endurance exercise training acts like estrogens in neutralizing increased gene expression of IL-6, IKKB, and NF-κB in liver of Ovx rats. Although this effect of exercise training in liver might be solely linked to the reduction of fat accumulation, it is important to recall that physical activity mediates anti-inflammatory effects in skeletal muscle and fat tissue (18). Regular exercise protects against diseases associated with chronic low-grade systemic inflammation and the long-term effect of exercise training may be ascribed to the anti-inflammatory response elicited by an acute bout of exercise (48).

TNF-α plays a central role in initiating and sustaining inflammation (49), while IL-10 demonstrates potent anti-inflammatory properties through inhibiting the production of various pro-inflammatory cytokines including IL-6 and TNF-α (50). In fact, Kaur *et al.* (51) showed that a proper balance between IL-10 and TNF-α rather than any of these individual cytokine responses is of physiological importance. Hashem *et al.* (52) reported that IL-10/TNF-α ratio is a convenient predictive biomarker for investigation of fatty liver of different grades including steatohepatitis and nonalcoholic fatty liver disease. The present increase in gene expression ratio of IL-10/TNF-α with E2 replacement as well as with endurance exercise training in Ovx animals, therefore, indicates an improvement in the status of the liver in both of these conditions.

An intriguing response related to inflammatory biomarkers in the present study is the observation that plasma CRP as well as CRP mRNA levels in liver decreased in Ovx

animals and re-increased with E2 replacement. A number of investigations have reported that hormone replacement therapy increases plasma CRP levels (53-55) especially in response to oral conjugated estrogens (56). Although it has been argued that rats are not an appropriate model to investigate the relationship between estrogen and CRP, Yang *et al.* showed that ovariectomy in rat reduces plasma CRP and that estrogen replacement raises the plasma CRP levels (57). It has been suggested that E2-mediated increase in CRP may not represent an up-regulation of pro-inflammatory response mediated by upstream cytokines but rather are related to a secondary mechanism (56). Nevertheless, in the present study, plasma and liver mRNA expression of CRP did not increase in endurance exercise trained Ovx rats. This indicates that whatever the clinical significance of the action of E2 on increasing CRP levels, this is not carried out in exercise trained rats.

In summary, results of present study indicate that exercise training acts as estrogen supplementation in properly regulating gene expressions of molecular markers involved in liver fat accumulation and bio-markers of sub-clinical inflammation in Ovx rats. On a clinical point of view, the present results reiterate the importance of exercise training as a tool to alleviate some of the metabolic consequences of low estrogenic status in post-menopausal women.

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References

1. Clark JM. Weight loss as a treatment for nonalcoholic fatty liver disease. J Clin Gastroenterol. 2006 Mar;40 Suppl 1:S39-43.

2. Volzke H, Schwarz S, Baumeister SE, Wallaschofski H, Schwahn C, Grabe HJ, et al. Menopausal status and hepatic steatosis in a general female population. Gut. 2007 Apr;56(4):594-5.

3. Kadowaki T, Hara K, Yamauchi T, Terauchi Y, Tobe K, Nagai R. Molecular mechanism of insulin resistance and obesity. Exp Biol Med (Maywood). 2003 Nov;228(10):1111-7.

4. Stefan N, Kantartzis K, Machann J, Schick F, Thamer C, Rittig K, et al. Identification and characterization of metabolically benign obesity in humans. Arch Intern Med. 2008 Aug 11;168(15):1609-16.

5. Messier V, Karelis AD, Robillard ME, Bellefeuille P, Brochu M, Lavoie JM, et al. Metabolically healthy but obese individuals: relationship with hepatic enzymes. Metabolism. Jan;59(1):20-4.

6. Pighon A, Paquette A, Barsalani R, Chapados NA, Yasari S, Doucet E, et al. Substituting food restriction by resistance training prevents liver and body fat regain in ovariectomized rats. Climacteric. 2009 Apr;12(2):153-64.

7. Paquette A, Shinoda M, Rabasa Lhoret R, Prud'homme D, Lavoie JM. Time course of liver lipid infiltration in ovariectomized rats: impact of a high-fat diet. Maturitas. 2007 Oct 20;58(2):182-90.

8. Deshaies Y, Dagnault A, Lalonde J, Richard D. Interaction of corticosterone and gonadal steroids on lipid deposition in the female rat. Am J Physiol. 1997 Aug;273(2 Pt 1):E355-62.

9. Picard F, Deshaies Y, Lalonde J, Samson P, Labrie C, Belanger A, et al. Effects of the estrogen antagonist EM-652.HCl on energy balance and lipid metabolism in ovariectomized rats. Int J Obes Relat Metab Disord. 2000 Jul;24(7):830-40.

10. Paquette A, Wang D, Jankowski M, Gutkowska J, Lavoie JM. Effects of ovariectomy on PPAR alpha, SREBP-1c, and SCD-1 gene expression in the rat liver. Menopause. 2008 Nov-Dec;15(6):1169-75.

11. Toda K, Takeda K, Akira S, Saibara T, Okada T, Onishi S, et al. Alternations in hepatic expression of fatty-acid metabolizing enzymes in ArKO mice and their reversal by the treatment with 17beta-estradiol or a peroxisome proliferator. J Steroid Biochem Mol Biol. 2001 Dec;79(1-5):11-7.

12. Paquette A, Chapados NA, Bergeron R, Lavoie JM. Fatty acid oxidation is decreased in the liver of ovariectomized rats. Horm Metab Res. 2009 Jul;41(7):511-5.

13. Gauthier MS, Couturier K, Latour JG, Lavoie JM. Concurrent exercise prevents high-fat-diet-induced macrovesicular hepatic steatosis. J Appl Physiol. 2003 Jun;94(6):2127-34.

14. Gauthier MS, Couturier K, Charbonneau A, Lavoie JM. Effects of introducing physical training in the course of a 16-week high-fat diet regimen on hepatic steatosis, adipose tissue fat accumulation, and plasma lipid profile. Int J Obes Relat Metab Disord. 2004 Aug;28(8):1064-71.

15. Corriveau P, Paquette A, Brochu M, Prud'homme D, Rabasa-Lhoret R, Lavoie JM. Resistance training prevents liver fat accumulation in ovariectomized rats. Maturitas. 2008 Mar 20;59(3):259-67.

16. Pighon A, Paquette A, Barsalani R, Chapados NA, Rabasa-Lhoret R, Yasari S, et al. Resistance training attenuates fat mass regain after weight loss in ovariectomized rats. Maturitas. 2009 Sep 20;64(1):52-7.

17. Pighon A, Barsalani R, Yasari S, Prud'homme D, Lavoie JM. Does exercise training prior to ovariectomy protect against liver and adipocyte fat accumulation in rats? Climacteric. 2009 Aug 3:1-12.

18. Bruunsgaard H. Physical activity and modulation of systemic low-level inflammation. J Leukoc Biol. 2005 Oct;78(4):819-35.

19. Esposito K, Giugliano D. The metabolic syndrome and inflammation: association or causation? Nutr Metab Cardiovasc Dis. 2004 Oct;14(5):228-32.

20. Cai D, Yuan M, Frantz DF, Melendez PA, Hansen L, Lee J, et al. Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. Nature medicine. 2005 Feb;11(2):183-90.

21. Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. J Clin Invest. 2006 Jul;116(7):1793-801.

22. Robertson MC, Owens RE, Klindt J, Friesen HG. Ovariectomy leads to a rapid increase in rat placental lactogen secretion. Endocrinology. 1984 May;114(5):1805-11.

23. El-Mas MM, Abdel-Rahman AA. Effects of long-term ovariectomy and estrogen replacement on clonidine-evoked reductions in blood pressure and hemodynamic variability. J Cardiovasc Pharmacol. 2004 May;43(5):607-15.

24. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985 Jul;28(7):412-9.

25. Barsalani R, Pighon A, Rabasa-Lhoret R, Yasari S, Lavoie JM. Liver of ovariectomized rats is resistant to resorption of lipids. Physiol Behav. 2008 Sep 3;95(1-2):216-21.

26. Heine PA, Taylor JA, Iwamoto GA, Lubahn DB, Cooke PS. Increased adipose tissue in male and female estrogen receptor-alpha knockout mice. Proc Natl Acad Sci U S A. 2000 Nov 7;97(23):12729-34.

27. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. J Clin Invest. 2005 May;115(5):1343-51.

28. Kohjima M, Enjoji M, Higuchi N, Kato M, Kotoh K, Yoshimoto T, et al. Re-evaluation of fatty acid metabolism-related gene expression in nonalcoholic fatty liver disease. Int J Mol Med. 2007 Sep;20(3):351-8.

29. Cohen P, Ntambi JM, Friedman JM. Stearoyl-CoA desaturase-1 and the metabolic syndrome. Curr Drug Targets Immune Endocr Metabol Disord. 2003 Dec;3(4):271-80.

30. Cohen P, Miyazaki M, Socci ND, Hagge-Greenberg A, Liedtke W, Soukas AA, et al. Role for stearoyl-CoA desaturase-1 in leptin-mediated weight loss. Science. 2002 Jul 12;297(5579):240-3.

31. Dentin R, Pegorier JP, Benhamed F, Foufelle F, Ferre P, Fauveau V, et al. Hepatic glucokinase is required for the synergistic action of ChREBP and SREBP-1c on glycolytic and lipogenic gene expression. J Biol Chem. 2004 May 7;279(19):20314-26.

32. Desvergne B, Michalik L, Wahli W. Be fit or be sick: peroxisome proliferator-activated receptors are down the road. Mol Endocrinol. 2004 Jun;18(6):1321-32.

33. Desvergne B, Wahli W. Peroxisome proliferator-activated receptors: nuclear control of metabolism. Endocr Rev. 1999 Oct;20(5):649-88.

34. Bjornstrom L, Sjoberg M. Mechanisms of estrogen receptor signaling: convergence of genomic and nongenomic actions on target genes. Mol Endocrinol. 2005 Apr;19(4):833-42.

35. Kelly MJ, Levin ER. Rapid actions of plasma membrane estrogen receptors. Trends Endocrinol Metab. 2001 May-Jun;12(4):152-6.

36. Evinger AJ, 3rd, Levin ER. Requirements for estrogen receptor alpha membrane localization and function. Steroids. 2005 May-Jun;70(5-7):361-3.

37. Revankar CM, Cimino DF, Sklar LA, Arterburn JB, Prossnitz ER. A transmembrane intracellular estrogen receptor mediates rapid cell signaling. Science. 2005 Mar 11;307(5715):1625-30.

38. D'Eon TM, Souza SC, Aronovitz M, Obin MS, Fried SK, Greenberg AS. Estrogen regulation of adiposity and fuel partitioning. Evidence of genomic and non-genomic regulation of lipogenic and oxidative pathways. J Biol Chem. 2005 Oct 28;280(43):35983-91.

39. Bajic VB, Tan SL, Chong A, Tang S, Strom A, Gustafsson JA, et al. Dragon ERE Finder version 2: A tool for accurate detection and analysis of estrogen response elements in vertebrate genomes. Nucleic Acids Res. 2003 Jul 1;31(13):3605-7.

40. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, et al. Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest. 2001 Oct;108(8):1167-74.

41. Winder WW, Thomson DM. Cellular energy sensing and signaling by AMP-activated protein kinase. Cell Biochem Biophys. 2007;47(3):332-47.

42. Paquette A, Wang D, Gauthier MS, Prud'homme D, Jankowski M, Gutkowska J, et al. Specific adaptations of estrogen receptor alpha and beta transcripts in liver and heart after endurance training in rats. Mol Cell Biochem. 2007 Dec;306(1-2):179-87.

43. Saengsirisuwan V, Pongseeda S, Prasannarong M, Vichaiwong K, Toskulkao C. Modulation of insulin resistance in ovariectomized rats by endurance exercise training and estrogen replacement. Metabolism. 2009 Jan;58(1):38-47.

44. Arkan MC, Hevener AL, Greten FR, Maeda S, Li ZW, Long JM, et al. IKK-beta links inflammation to obesity-induced insulin resistance. Nat Med. 2005 Feb;11(2):191-8.

45. Chen H. Cellular inflammatory responses: novel insights for obesity and insulin resistance. Pharmacol Res. 2006 Jun;53(6):469-77.

46. Kireev RA, Tresguerres AC, Garcia C, Borras C, Ariznavarreta C, Vara E, et al. Hormonal regulation of pro-inflammatory and lipid peroxidation processes in liver of old ovariectomized female rats. Biogerontology. 2009 Jul 26.

47. Hamilton KL, Lin L, Wang Y, Knowlton AA. Effect of ovariectomy on cardiac gene expression: inflammation and changes in SOCS gene expression. Physiol Genomics. 2008 Jan 17;32(2):254-63.

48. Petersen AM, Pedersen BK. The anti-inflammatory effect of exercise. J Appl Physiol. 2005 Apr;98(4):1154-62.

49. Ksontini R, MacKay SL, Moldawer LL. Revisiting the role of tumor necrosis factor alpha and the response to surgical injury and inflammation. Arch Surg. 1998 May;133(5):558-67.

50. Bolger AP, Sharma R, von Haehling S, Doehner W, Oliver B, Rauchhaus M, et al. Effect of interleukin-10 on the production of tumor necrosis factor-alpha by peripheral

blood mononuclear cells from patients with chronic heart failure. Am J Cardiol. 2002 Aug 15;90(4):384-9.

51. Kaur K, Sharma AK, Dhingra S, Singal PK. Interplay of TNF-alpha and IL-10 in regulating oxidative stress in isolated adult cardiac myocytes. J Mol Cell Cardiol. 2006 Dec;41(6):1023-30.

52. Hashem RM, Mahmoud MF, El-Moselhy MA, Soliman HM. Interleukin-10 to tumor necrosis factor-alpha ratio is a predictive biomarker in nonalcoholic fatty liver disease: interleukin-10 to tumor necrosis factor-alpha ratio in steatohepatitis. Eur J Gastroenterol Hepatol. 2008 Oct;20(10):995-1001.

53. Pradhan AD, Manson JE, Rossouw JE, Siscovick DS, Mouton CP, Rifai N, et al. Inflammatory biomarkers, hormone replacement therapy, and incident coronary heart disease: prospective analysis from the Women's Health Initiative observational study. JAMA. 2002 Aug 28;288(8):980-7.

54. Miller AP, Chen YF, Xing D, Feng W, Oparil S. Hormone replacement therapy and inflammation: interactions in cardiovascular disease. Hypertension. 2003 Oct;42(4):657-63.

55. Ridker PM, Hennekens CH, Rifai N, Buring JE, Manson JE. Hormone replacement therapy and increased plasma concentration of C-reactive protein. Circulation. 1999 Aug 17;100(7):713-6.

56. Georgiadou P, Sbarouni E. Effect of hormone replacement therapy on inflammatory biomarkers. Adv Clin Chem. 2009;47:59-93.

57. Yang SX, Diaz Padilla N, Zhu Q, Ma XM, Sasso D, Prestwood K, et al. Estrogen replacement raises rat CRP without evidence of complement activation. Endocr Res. 2005;31(2):121-32.

Table 1. Oligonucleotide primers used for quantitative real-time polymerase chain reaction.

Genes	Accession no	Sense primer (5′-3′)	Antisense primer (5′-3′)
SREBP-1c	XM_213329	TACAGCGTGGCTGGGAAC	GGCTGAGCGATACAGTTCAA
SCD-1	NM_139192	GCCCTGTACGGGATCACA	CCCAGGGCACTGATAAGGTA
ChREBP	NM_133552	AATCCCAGCCCCTACACC	CTGGGAGGAGCCAATGTG
ACC	NM_022193	ACAGAGATGGTGGCTGATGTC	GATCCCCATGGCAATCTG
PPAR-α	NM_013196	TCGGAGGGCTCTGTCATC	CATCTGTACTGGTGGGGACA
PGC-1α	NM_031347	GAAGCGGGAGTCTGAAAGG	GTAAATCACACGGCGCTCTT
CRP	NM_017096	CTTCTCTCAGGCTTTTGGTCA	GCTTCCAGTGGCTTCTTTGA
IKKB	NM_053355	GAGAGCGTCAGCTGTGTCC	CCCCACACTTTCCTCATCTG
IL-6	NM_012589	CCCTTCAGGAACAGCTATGAA	ACAACATCAGTCCCAAGAAGG
TNF-α	NM_012675	GCCTCTTCTCATTCCTGCTC	GAGCCCATTTGGGAACTTCT
IL-10	NM_012854	GCTCAGCACTGCTATGTTGC	AATGGCCTTTGCTGGTCTT
GAPDH	NM_017008	CCCTCTGGAAAGCTGTGG	AGTGGATGCAGGGATGATG

SREBP-1c: sterol regulatory element-binding protein-1c; SCD-1: stearoyl CoA desaturase-1; ChREBP: carbohydrate response element-binding protein; ACC: acetyl-CoA carboxylase; PPAR-α: peroxisome proliferator activated receptor-α; PGC-1α: Peroxisome proliferator-activated receptor-γ coactivator 1α; CRP: C-reactive protein; IKKB: inhibitor of kappa B kinase beta; IL-6: interleukin 6; TNF-α: tumor necrosis factor-α; IL-10: interleukin 10; GADPH: glyceraldehyde-3-phosphate dehydrogenase.

Table 2. Effects of ovariectomy, estrogen replacement, and exercise training on anthropometric and physiological parameters.

	Sham	**Ovx**	**OvxE2**	**OvxTr**
Body weight (g)	345±13	388±12^{abb}	314±5	357±13^{b}
Energy intake (kj/day)	333±11	378±11^{aabbc}	331±9	341±9
Mesenteric fat weight (g)	9.52±0.9	13.6±1.2^{aabbcc}	8.72±0.6	7.83±0.5
Retroperitoneal fat weight (g)	5.69±0.8	8.69±1^{aabbcc}	5.67±0.6	5.0±0.4
Uterus weight (mg)	602±51	123±9^{aabb}	774±64^{aa}	123±8^{aabb}
Leg muscles weight (g/100gBW)	0.62±0.02	0.6±0.02^{bbcc}	0.67±0.02	0.71±0.02^{aa}
Femur weight (g/100gBW)	0.21±0.007	0.18±0.01^{abbcc}	0.24±0.009^{aa}	0.22±0.007
Plasma insulin (pM)	263±22	387±52 $^{(abc)*}$	231±34	250±50
Plasma glucose (mM)	8.62±0.3	10.5±0.4^{aabb}	6.83±0.3^{aacc}	9.59±0.3^{a}
HOMA-IR (mIU/l)	16.8±1.5	29.8±4.5^{aabbc}	11.9±1.8	17.7±3.7
Plasma Leptin (ng/ml)	8.85±1.1	13.6±1.3^{aabbcc}	8.81±0.9	6.63±0.8

[a] Significantly different from Sham, $P < 0.05$, **[aa]** $P < 0.01$. **[b]** significantly different from OvxE2, $P < 0.05$, **[bb]** $P < 0.01$. **[c]** significantly different from OvxTr, $P < 0.05$, **[cc]** $P < 0.01$. $^{(abc)*}$: ($P = 0.059$). Values are mean ± S.E., n = 6-8 rats/group.

Sham: sham-operated; Ovx: ovariectomized; OvxE2: ovariectomized with 17β-estradiol supplementation; OvxTr: ovariectomized + endurance exercise training.

Figure legends

Figure 1. Liver triacyleglycerol (TAG) (A) and sterol regulatory element-binding protein-1c (SREBP-1c) mRNA (B) in sham-operated (Sham), ovariectomized (Ovx), ovariectomized with 17β-estradiol supplementation (OvxE2), and ovariectomized + endurance exercise training (OvxTr) rats. ^{aa} Significantly different from Sham, $P < 0.01$. ^{b} significantly different from OvxE2, $P < 0.05$, ^{bb} $P < 0.01$. ^{c} significantly different from OvxTr, $P < 0.05$, ^{cc} $P < 0.01$. Values are mean ± S.E., n = 6-8 rats/group.

Figure 2. Hepatic lipogenic mRNA and protein abundance in sham-operated (Sham), ovariectomized (Ovx), ovariectomized with 17β-estradiol supplementation (OvxE2), and ovariectomized + endurance exercise training (OvxTr) rats. ^{aa} Significantly different from Sham, $P < 0.01$. ^{b} significantly different from OvxE2, $P < 0.05$, ^{bb} $P < 0.01$. ^{cc} significantly different from OvxTr, $P < 0.01$. Values are mean ± S.E., n = 6-8 rats/group.

SCD-1: stearoyl CoA desaturase-1; ChREBP: carbohydrate response element-binding protein; ACC: acetyl-CoA carboxylase.

Figure 3. Hepatic lipid oxidative mRNA abundance in sham-operated (Sham), ovariectomized (Ovx), ovariectomized with 17β-estradiol supplementation (OvxE2), and ovariectomized + endurance exercise training (OvxTr) rats. ^{aa} Significantly different from Sham, $P < 0.01$. ^{bb} significantly different from OvxE2, $P < 0.01$. ^{c} significantly different from OvxTr, $P < 0.05$. Values are mean ± S.E., n = 6-8 rats/group.

PPAR-α: peroxisome proliferator activated receptor-α; PGC-1α: Peroxisome proliferator-activated receptor-γ coactivator 1α.

Figure 4. Hepatic gene expression of interleukin 6 (IL-6) (A), inhibitor of kappa B kinase beta (IKKB) (B), and the protein abundance of nuclear factor-kappa B (NF-κB) (C)

in sham-operated (Sham), ovariectomized (Ovx), ovariectomized with 17β-estradiol supplementation (OvxE2), and ovariectomized + endurance exercise training (OvxTr) rats. [a] Significantly different from Sham, $P < 0.05$, [aa] $P < 0.01$. [bb] significantly different from OvxE2, $P < 0.01$. [c] significantly different from OvxTr, $P < 0.05$, [cc] $P < 0.01$. Values are mean ± S.E., n = 6-8 rats/group.

Figure 5. Hepatic gene expression of tumor necrosis factor-α (TNF-α) (A), interleukin 10 (IL-10) (B), and IL-10/TNF-α ratio (C) in sham-operated (Sham), ovariectomized (Ovx), ovariectomized with 17β-estradiol supplementation (OvxE2), and ovariectomized + endurance exercise training (OvxTr) rats. [b] significantly different from OvxE2, $P < 0.05$. [c] significantly different from OvxTr, $P < 0.05$. Values are mean ± S.E., n = 6-8 rats/group.

Figure 6. Plasma C-reactive protein (CRP) levels (A) and hepatic gene expression of CRP (B) in sham-operated (Sham), ovariectomized (Ovx), ovariectomized with 17β-estradiol supplementation (OvxE2), and ovariectomized + endurance exercise training (OvxTr) rats. [aa] Significantly different from Sham, $P < 0.01$. [bb] significantly different from OvxE2, $P < 0.01$. [cc] significantly different from OvxTr, $P < 0.01$. Values are mean ± S.E., n = 6-8 rats/group.

Figure 1.

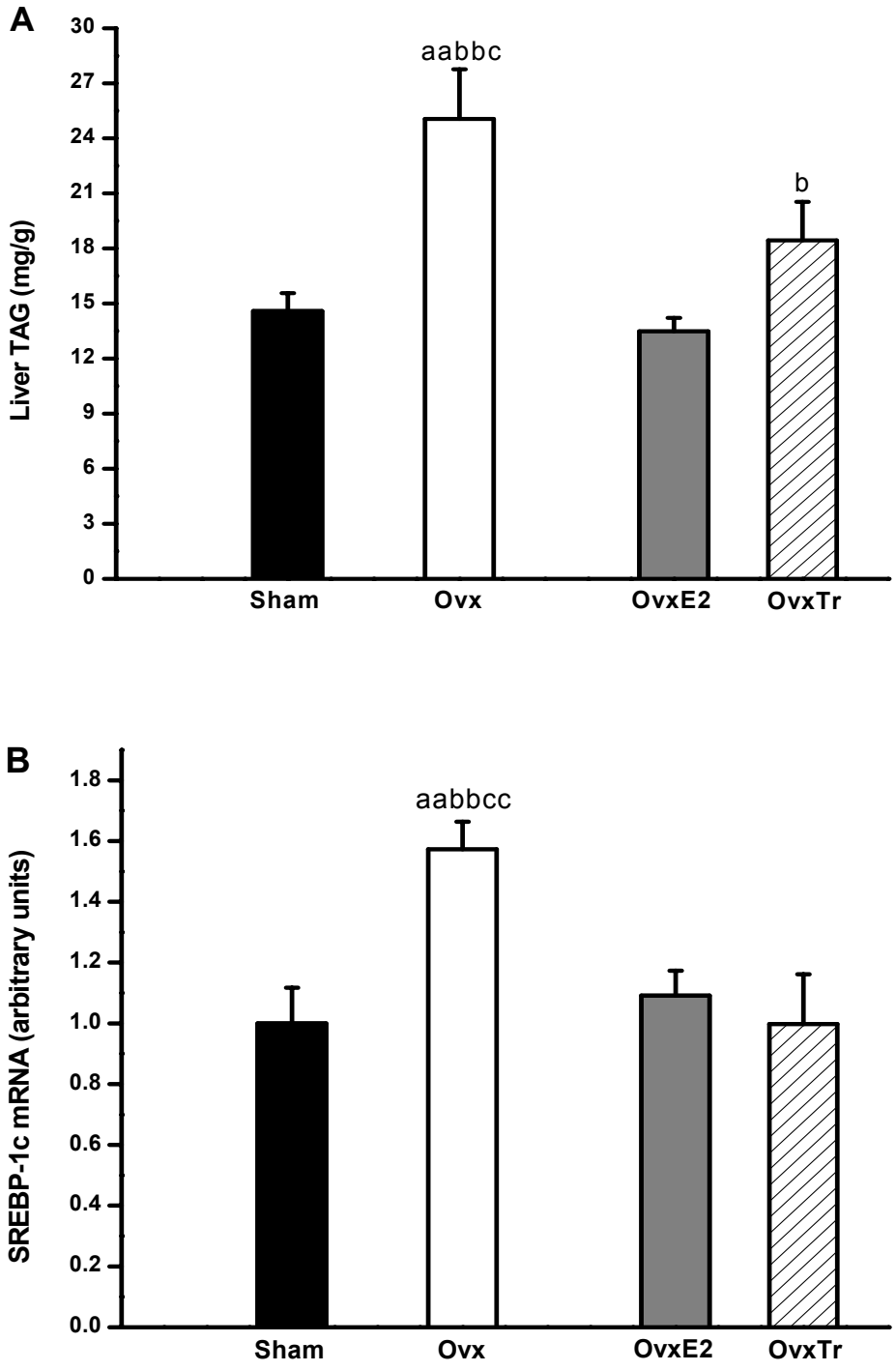
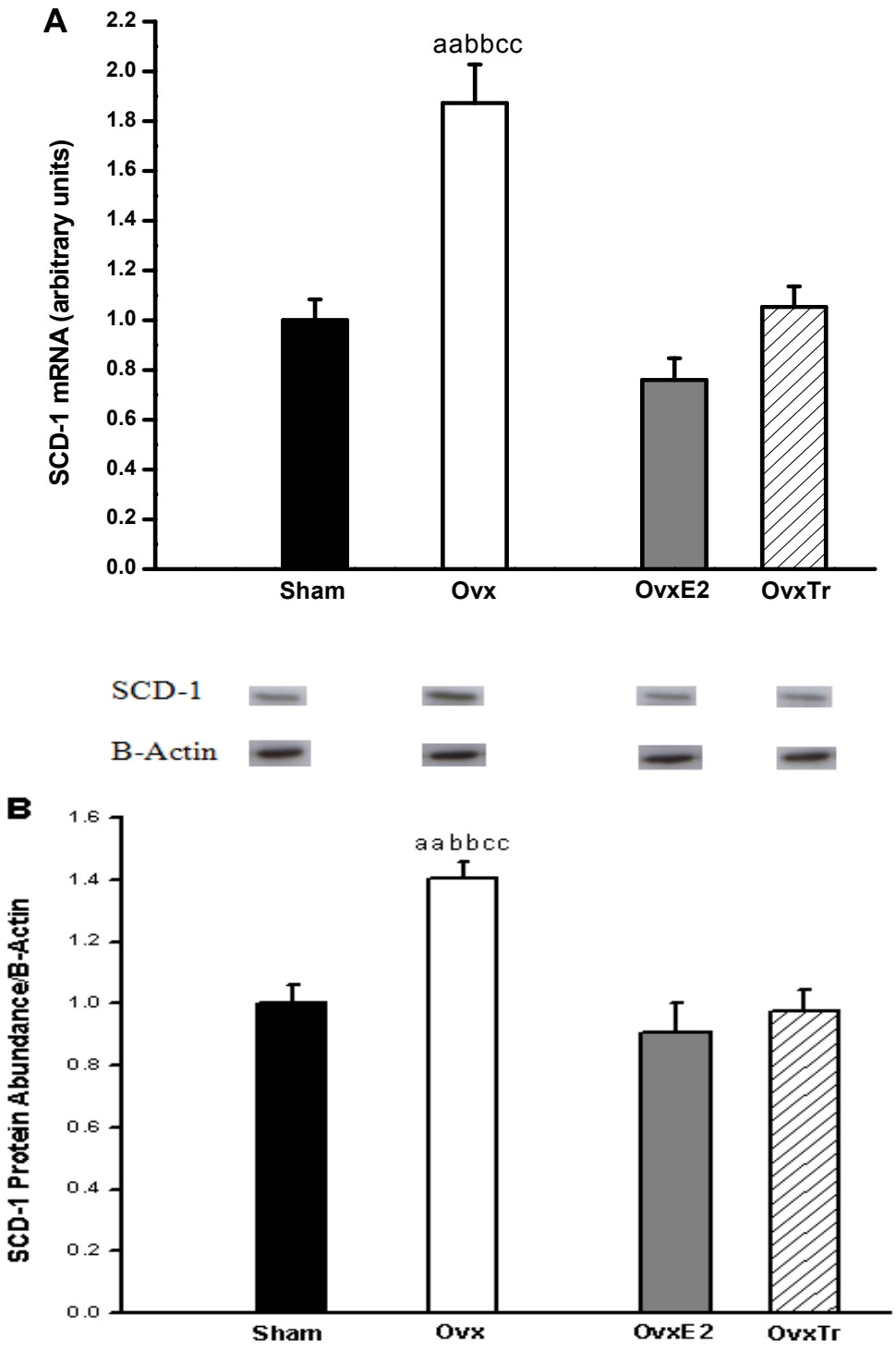
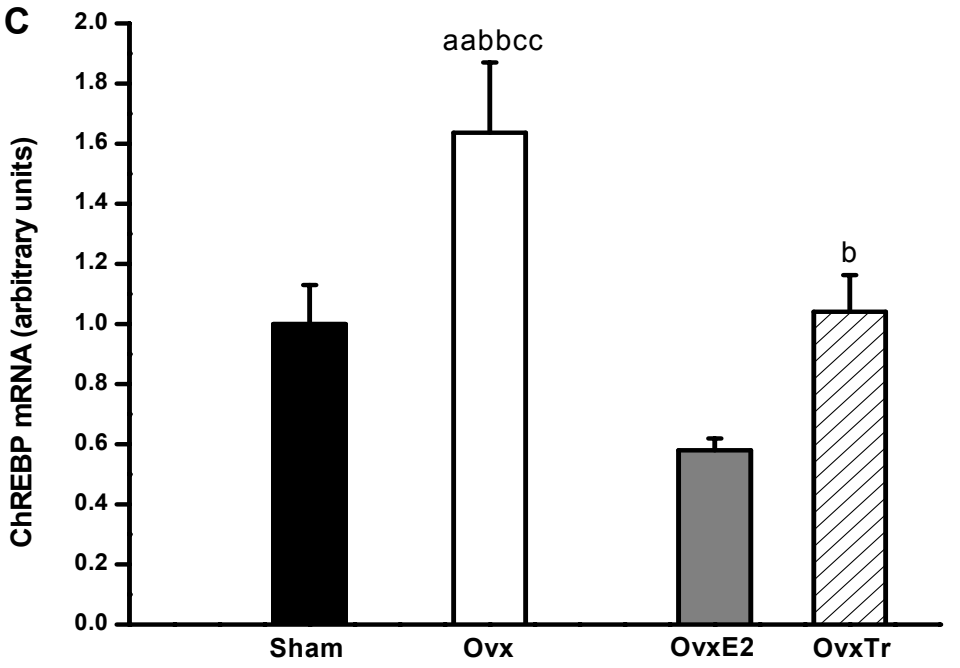

A
Liver TAG (mg/g)
30
27
24
21
18
15
12
9
6
3
0
aabbc
b
Sham
Ovx
OvxE2
OvxTr
B
SREBP-1c mRNA (arbitrary units)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
aabbcc
Sham
Ovx
OvxE2
OvxTr

Figure 2.


A
SCD-1 mRNA (arbitrary units)
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
aabbcc
Sham
Ovx
OvxE2
OvxTr
SCD-1
B-Actin
B
SCD-1 Protein Abundance/B-Actin
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
aabbcc
Sham
Ovx
OvxE2
OvxTr


C
ChREBP mRNA (arbitrary units)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
aabbcc
b
Sham
Ovx
OvxE2
OvxTr

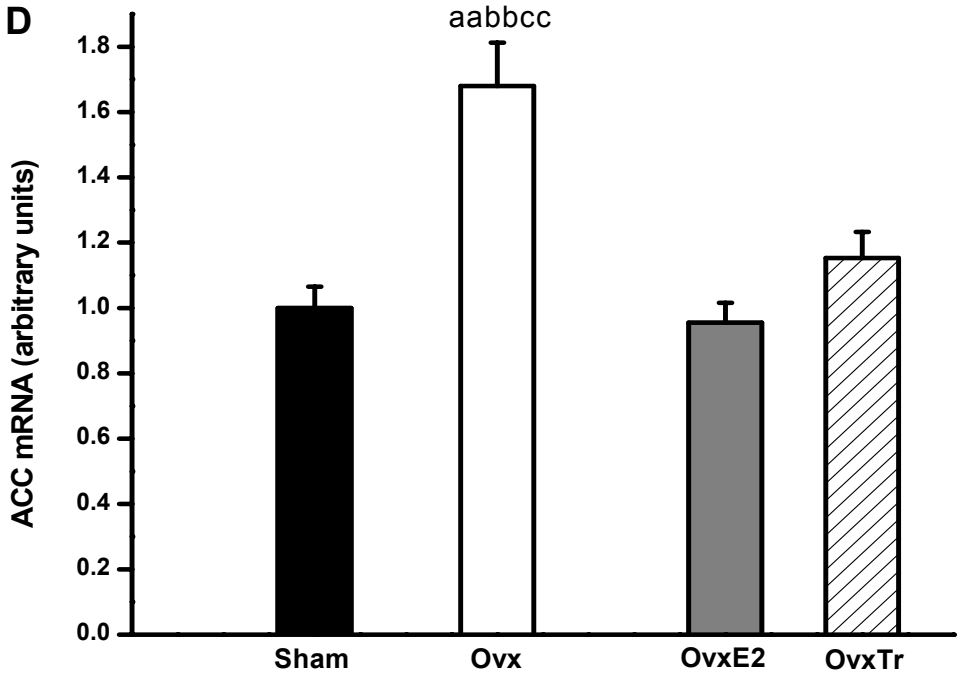

D
ACC mRNA (arbitrary units)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
aabbcc
Sham
Ovx
OvxE2
OvxTr

Figure 3.

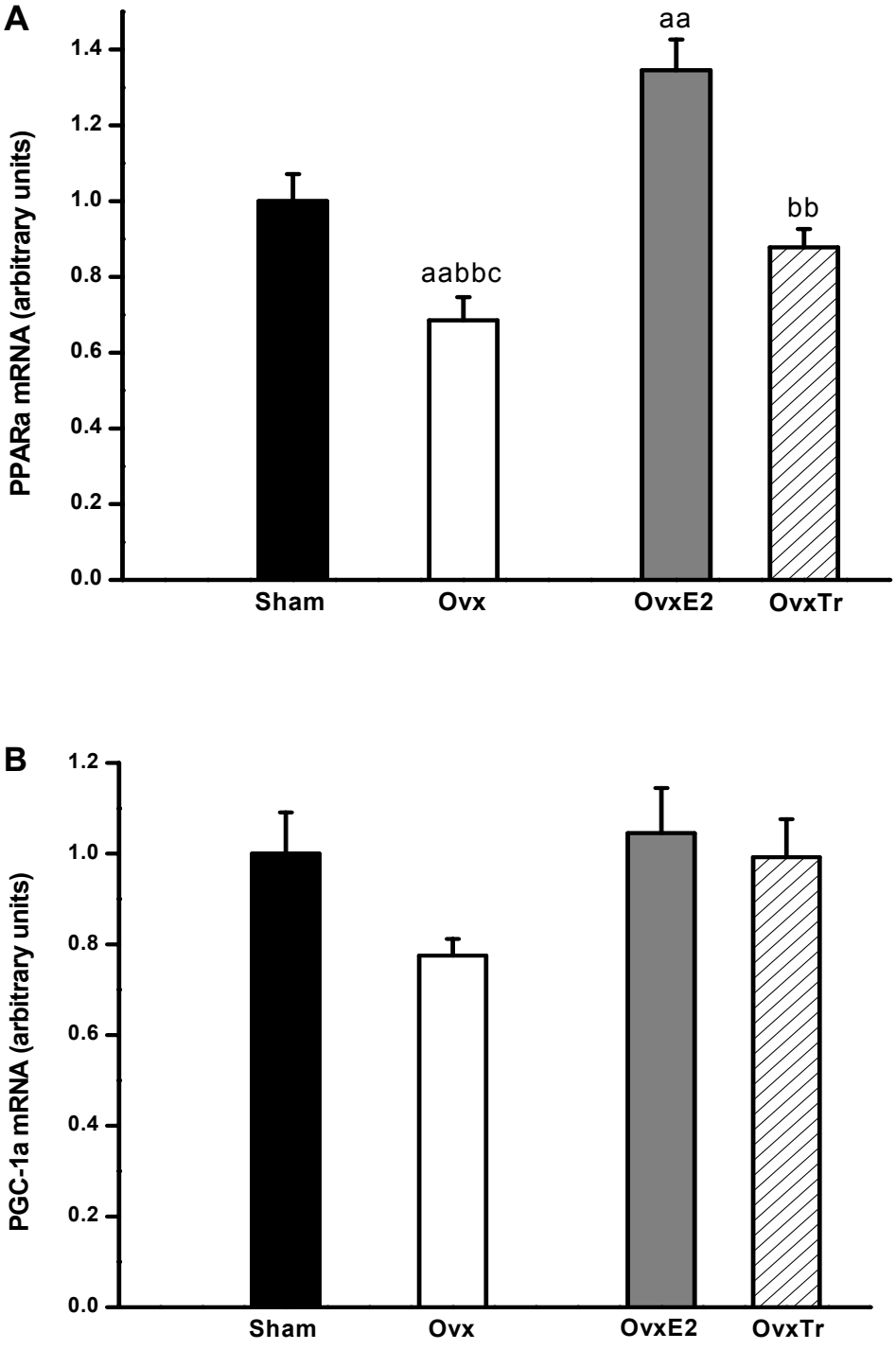

A
PPARa mRNA (arbitrary units)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
aa
bb
aabbc
Sham
Ovx
OvxE2
OvxTr
B
PGC-1a mRNA (arbitrary units)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Sham
Ovx
OvxE2
OvxTr

Figure 4.

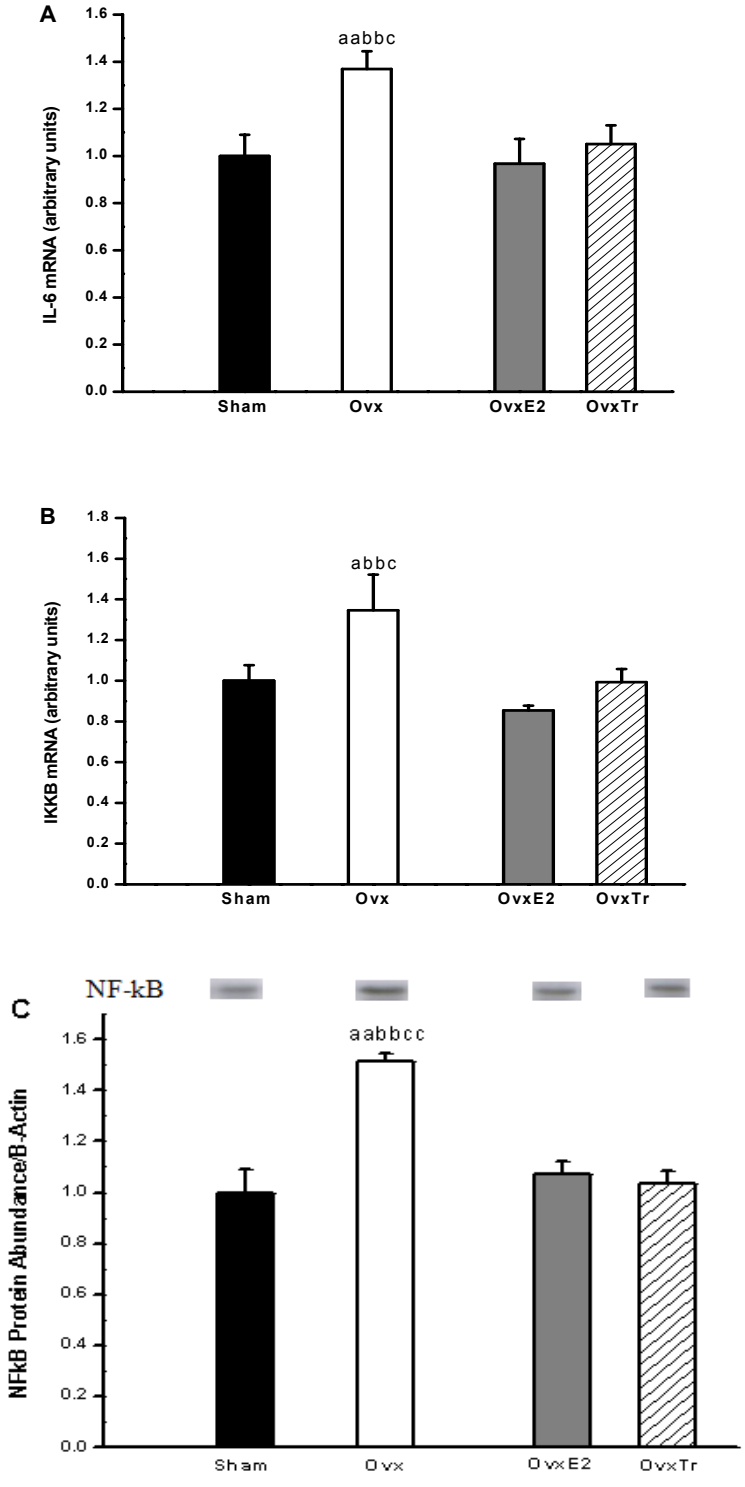

A
IL-6 mRNA (arbitrary units)
aabbc
Sham
Ovx
OvxE2
OvxTr
B
IKKB mRNA (arbitrary units)
abbc
Sham
Ovx
OvxE2
OvxTr
C
NF-kB
NFkB Protein Abundance/B-Actin
aabbcc
Sham
Ovx
OvxE2
OvxTr

Figure 5.

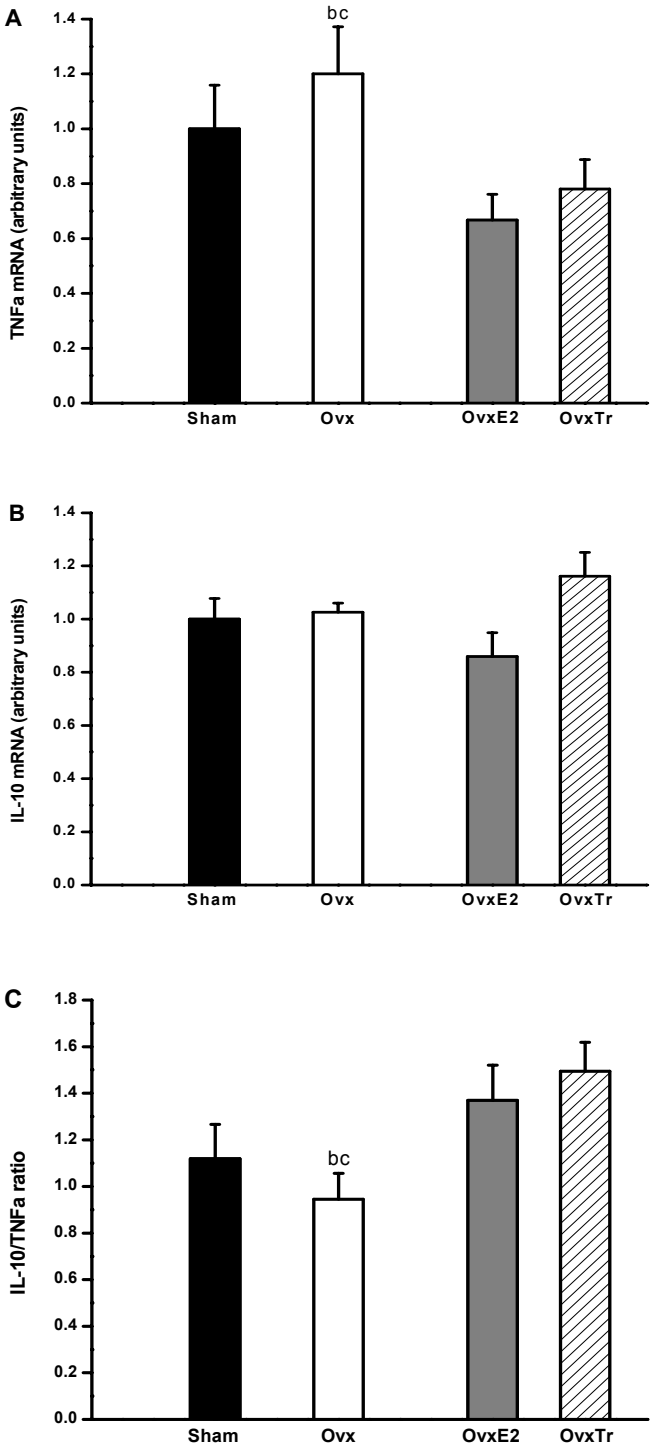
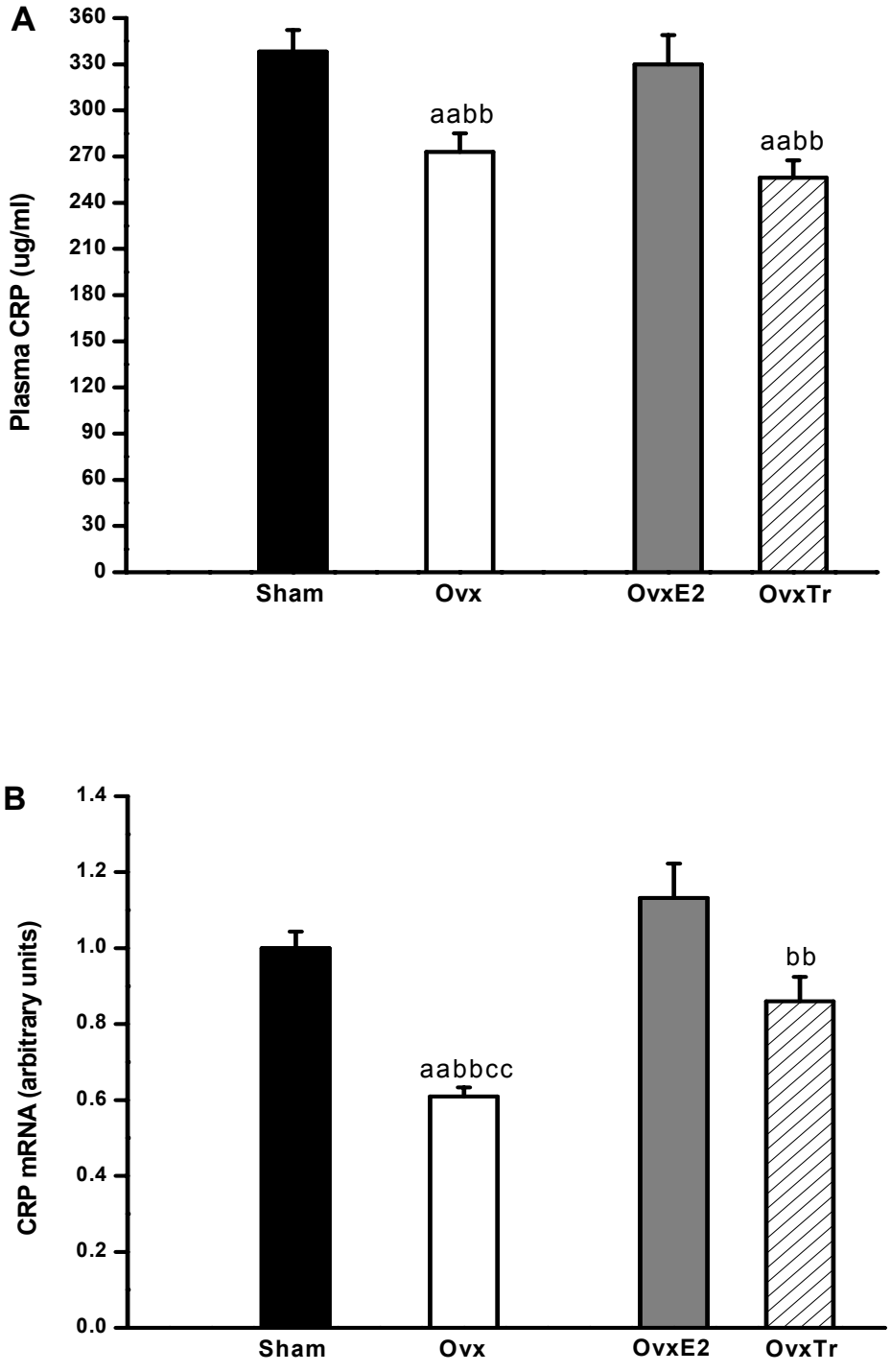

A
TNFa mRNA (arbitrary units)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
bc
Sham
Ovx
OvxE2
OvxTr
B
IL-10 mRNA (arbitrary units)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Sham
Ovx
OvxE2
OvxTr
C
IL-10/TNFa ratio
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
bc
Sham
Ovx
OvxE2
OvxTr

Figure 6.


A
Plasma CRP (ug/ml)
360
330
300
270
240
210
180
150
120
90
60
30
0
aabb
aabb
Sham
Ovx
OvxE2
OvxTr
B
CRP mRNA (arbitrary units)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
aabbcc
bb
Sham
Ovx
OvxE2
OvxTr

Chapter 6: General discussion and conclusion

The general objective of the studies presented in this thesis was to provide information regarding treatment and prevention of hepatic steatosis development and adipocyte fat accumulation in the estrogen deficient animals using lifestyle modifications (i.e. exercise training) as an alternative strategy for hormone replacement therapy. Since prospective studies assessing the physiological and biochemical effects of exercise in humans is difficult to carry out in controlled settings [de Lemos, Reis et al. 2007], we used an ovariectomized animal model that provided us with an appropriate research tool to mimic the postmenopausal hormonal state. If confirmed in human studies, the observations in the presented studies would be clinically relevant to the growing menopausal population in a society defined by energy overconsumption. However, we acknowledge the fact that the conclusions of our studies are limited to the employed experimental model. Human aging/onset of menopause implies a continuum of estrogen changes. Our model of ovariectomy is an aggressive method of estrogen withdrawal and may result in changes in metabolic profile and gene expression that may differ from those imposed by menopause in humans. Nevertheless, given that the risk of all causes of mortality associated with surgical ovariectomy in women younger than 45 year-old is increased [Rocca, Grossardt et al. 2006], we believe that our model and experiments still provide information reasonably relevant to basic research on estrogen related changes in metabolic profile and gene expression. In support of this, Barlet *et al.* and Kalu reported that the ovariectomized rat model is suitable for studying problems that are relevant to postmenopausal bone loss [Kalu 1991; Barlet, Coxam et al. 1994].

All Ovx animals in the four studies showed lower uterus weight and higher food intake, body weight and fat pads weight than Sham rats indicating typical morphologic changes associated with ovariectomy [Lemieux, Picard et al. 2003; Paquette, Shinoda et al. 2007] thus confirming quality of total ovariectomy in our studies. Moreover, E2 supplementation in the last two studies totally prevented these Ovx-induced changes confirming that the observed effects in Ovx rats were indeed related to estrogen shortage.

The four original studies presented in this thesis provided information regarding the treatment and prevention of ovariectomy-induced fat accumulation in adipocytes and hepatocytes by lifestyle modifications including restrictive diets and physical activity in the form of resistance and endurance exercise training. Since in recent years hepatic steatosis was recognized as a hepatic component of the metabolic syndrome, we focused on liver lipid accretion after ovariectomy. The data from the first two studies support the concept that resistance training might provide a successful alternative or complement to chronic restrictive diet for preventing body weight gain, peripheral fat accumulation, and liver lipid infiltration following estrogen withdrawal and for avoiding their relapse in Ovx rats. This positive effect of resistance training on liver TAG followed the changes in fat mass accumulation in Ovx rats. Therefore, it is reasonable to conclude that liver fat infiltration is largely associated to adipose tissue fat accumulation; highlighting the dominant central properties of estrogen withdrawal compared to intra-hepatic effects. In this regard, food restriction in ovariectomized animals would most probably reduce the amount of lipids taken up by the liver. On the other hand, resistance training can increase the energy expenditure counteracting the central effects of estrogen withdrawal. Nevertheless, it is possible that resistance training act intra-hepatically invigorating metabolic pathways in liver common to estrogens such as stimulating lipid oxidation inside the liver itself, in addition to increasing energy expenditure. As mentioned in the first study, the appropriateness of the present resistance training program needs to be addressed. Since it is difficult to use a resistance training program in rats without mobilizing several muscle groups, one may discuss that our results in the first two studies might be partially originated from aerobic adaptations of employed exercise program. In humans, a 6-month resistance training program in relatively healthy postmenopausal subjects did not contribute to improving the metabolic profile [Brochu, Malita et al. 2009]. Although the comparison between animal and human studies is difficult, the discrepancy between the two studies may be due to the intensity of employed programs and the better control of the training regimen in our animal studies.

We conducted our third study in an attempt to address the question whether women who exercise regularly during their reproductive period of life are protected against the deleterious metabolic effects of menopause. Contrary to our first two studies, we addressed this question using endurance exercise training. Using the Ovx animal model, we were able to test a complete design of trained and untrained animals before and after withdrawal of estrogens. The data from this study 1) confirmed our previous observations that ovariectomy leads to increased adipose tissue mass and liver TAG accretion that was prevented in E2-supplemented rats; 2) showed that there is no protective effect of a previous exercise-training state on ovariectomy-induced liver and adipocyte fat accumulation if rats remain sedentary after ovariectomy; 3) similarly to estrogen supplementation, endurance exercise training conducted concomitantly with estrogen induction has protective effect on ovariectomy-induced liver and adipocytes fat accumulation, whether or not rats were previously trained. Our observation that endurance exercise training has a strong influence on lowering body fat accumulation following a decrease in estrogen levels was recently confirmed by a research group in Germany [Zoth, Weigt et al. 2010]. Again, it is likely that mechanisms involved in exercise effects counteracted the central effects of estrogen withdrawal (increased energy expenditure) and induced intra-hepatic metabolic adaptations (decreased lipogenesis and increased hepatic lipid oxidation). More importantly, in this study we found that the liver lipid infiltration was the only measured parameter in which the training status affected only the ovariectomized rats (not the Sham and OvxE2 animals) increasing our interest about the intra-hepatic effects of estrogens and exercise training in Ovx rats. The role of estrogen in the regulation of energy homeostasis in females is well recognized. On the other hand, it is also well acknowledged that exercise training has beneficial effects on the metabolic syndrome. Considering the fact that pair-feeding in Ovx rats does not completely prevent fat accumulation in liver [unpublished data from our lab], we hypothesized that Ovx-induced hepatic fat accumulation cannot be totally attributed to the central effects of estrogen withdrawal and our data in the fourth study open a new avenue for the

investigation of potential mechanisms that might explain benefits of exercise in postmenopausal women.

Supporting the recent reports of Paquette *et al.* [Paquette, Wang et al. 2008; Paquette, Chapados et al. 2009], data of the fourth study confirms that withdrawal of estrogen affects hepatic pathways of lipid synthesis and oxidation. We showed that mRNA levels of SREBP1-c and ChREBP which are known as the two major transcription factors in hepatic lipogenesis and the gene expression of their downstream enzyme proteins SCD-1 and ACC are up-regulated in Ovx animals; while gene expression of PPAR-α, the key transcriptional regulator of lipid oxidation in liver of Ovx rats was decreased. In this last study, we provided important information regarding the similar effects of exercise training and estrogen replacement therapy in ovariectomized animals. Our original findings show that endurance exercise training, similarly to E2 supplementation, counteracted these hepatic molecular disturbances by cancelling the increase in SREBP-1c, SCD-1, ChREBP, and ACC transcripts; while re-increasing gene expression of PPAR-α in Ovx rats. These observations suggest that exercise training acts as estrogen supplementation in properly regulating gene expressions of molecular markers involved in liver fat accumulation in ovariectomized rats. The moderately short duration of our endurance exercise training program (5 weeks) may be a limitation of our study. To comprehend the full effect of exercise training on Ovx-induced liver lipid accumulation, future studies should consider extending exercise training for longer periods and controlling the central effects of estrogen withdrawal such as using pair-feeding model. Moreover, the intensity of exercise training used in the present studies can be considered as substantial. Moderating the intensity of exercise training program in future studies will allow relating the results to the clinical researches in postmenopausal women.

Since in recent years metabolic consequences of increased ectopic fat are associated with sub-clinical inflammation, we hypothesized that gene expressions of hepatic inflammatory bio-markers would be changed in Ovx animals. Results of our fourth study showed that mRNA levels of IL-6 and IKKB as well as protein content of NF-κB were

increased in liver of Ovx rats. As for metabolic markers, E2 supplementation alone or exercise training alone neutralized these elevated gene expressions. Moreover, we observed an increase in the ratio of IL-10/TNF-α gene expressions with E2 supplementation as well as with exercise training in Ovx rats. Although we are the first, to our knowledge, to put forward the concept that exercise training has an estrogenic-like effect on the improvement of pre-clinical inflammatory status in the liver of Ovx rats, it seems that further research is warranted to reach firm conclusion on the effect of exercise training on Ovx-hepatic-steatosis-induced sub-clinical inflammation in postmenopausal hormonal state.

Taken together, our results introduce the concept that exercise training can compensate estrogenic actions in the estrogen deficit context, at least to a certain extent of metabolic consequences. However, identification of mechanisms such as activation of estrogen receptors and downstream pathways stimulated by exercise training will permit to explore more details concerning this concept. As indicated by the results of this thesis, it seems that similarly to estrogen supplementation, exercise training prevents lipid accumulation in the liver of Ovx rats possibly through proper regulation of key intra-hepatic molecules implicated in lipogenesis and lipid oxidation; and/or through its secondary effects on lowering lipid storage in adipocytes (Table 6).

Table 6. Estrogenic-like effects of exercise training on metabolic and inflammatory bio-markers in Ovx rats.

Intra-hepatic Effects	Secondary Effects
Decreased lipogenesis • ↓ SREBP-1c mRNA • ↓ ChREBP mRNA • ↓ SCD-1 • ↓ ACC mRNA Increased lipid oxidation • ↑ PPAR-α mRNA Improved inflammatory bio-markers • ↓ IL-6 mRNA • ↓ IKKB mRNA • ↓ NF-κB • ↓ TNF-α mRNA • ↑ IL-10/ TNF-α mRNA	Most possibly increased energy expenditure Improved insulin sensitivity and lipid profile Decreased intra-abdominal and subcutaneous fat pads

See list of abbreviations for meaning of acronyms.

Conclusion

The results of the studies presented in this thesis complement previous findings regarding the central (increased intra-abdominal fat) and intra-hepatic effects (pathways of liver lipogenesis and lipid oxidation) of estrogen withdrawal favoring the accumulation of TAG in hepatocytes of Ovx rats. Our results clearly indicate that exercise training (either in the form of resistance or endurance) has a significant impact on ovariectomy-induced deleterious metabolic effects including reducing fat accumulation in liver and adipocytes. In addition, we showed for the first time that exercise training in Ovx rats seems to stimulate estrogenic-like effects on the expression of genes involved in lipid accumulation and sub-clinical inflammation in the liver. This is of importance knowing the fact that two-thirds of postmenopausal women are overweight and since utilization of hormone replacement therapy is under debate. Moreover, this thesis opens new avenue in designing clinical studies in women by promoting physical activity.

From a clinical point of view, results of this PhD work confirm the recommendation of The Study of Women's Health Across the Nation which suggests that ''not only do women who enter menopause transition (midlife) with higher level of physical activity and maintain that level weigh less to begin with and gain less weight over time, but women who increase their level of activity in midlife, regardless of where they start from, also gain less weight'' [Sternfeld, Wang et al. 2004]. Moreover, the results of the first two studies suggest that the application of resistance training can play a role to minimize the deleterious effects of menopause and may constitute a positive strategy to reduce body weight and fat mass relapse in postmenopausal women. These results are promising for health care staff providing advice to postmenopausal women for lifestyle changes that reduce the risks of insulin resistance, cardiovascular and coronary heart disease, and diabetes [Frank, Sorensen et al. 2005]. From a systemic review of randomized controlled exercise trials in postmenopausal women, it was recommended that early postmenopausal women could benefit from 30 minutes of daily moderate walking in one or three bouts combined with a resistance training program twice a week [Asikainen, Kukkonen-Harjula et al. 2004]. This

review indicates that such exercise training is likely to preserve normal weight and increase cardiovascular capacity, and improve disorders of lipid and carbohydrate metabolism.

Chapter 7: References

(1992). Methods for voluntary weight loss and control. NIH Technology Assessment Conference Panel. *Ann Intern Med* 116(11): 942-949.

(2006). Hormones and cardiovascular health in women. *Hum Reprod Update* 12(5): 483-497.

Abramson, J. L. and V. Vaccarino (2002). Relationship between physical activity and inflammation among apparently healthy middle-aged and older US adults. *Arch Intern Med* 162(11): 1286-1292.

Akira, S. and T. Kishimoto (1992). IL-6 and NF-IL6 in acute-phase response and viral infection. *Immunol Rev* 127: 25-50.

Akira, S., T. Taga, et al. (1993). Interleukin-6 in biology and medicine. *Adv Immunol* 54: 1-78.

Alexander, J. and M. Clearfield (2006). Cardiovascular disease after menopause: a growing epidemic. *Minerva Ginecol* 58(1): 35-40.

Alkhouri, N., T. A. Tamimi, et al. (2009). The Inflamed Liver and Atherosclerosis: A Link Between Histologic Severity of Nonalcoholic Fatty Liver Disease and Increased Cardiovascular Risk. *Dig Dis Sci*.

Angulo, P. (2002). Nonalcoholic fatty liver disease. *N Engl J Med* 346(16): 1221-1231.

Arabi, A., P. Garnero, et al. (2003). Changes in body composition during post-menopausal hormone therapy: a 2 year prospective study. *Hum Reprod* 18(8): 1747-1752.

Arkan, M. C., A. L. Hevener, et al. (2005). IKK-beta links inflammation to obesity-induced insulin resistance. *Nat Med* 11(2): 191-198.

Asikainen, T. M., K. Kukkonen-Harjula, et al. (2004). Exercise for health for early postmenopausal women: a systematic review of randomised controlled trials. *Sports Med* 34(11): 753-778.

Asikainen, T. M., S. Miilunpalo, et al. (2003). Walking trials in postmenopausal women: effect of low doses of exercise and exercise fractionization on coronary risk factors. *Scand J Med Sci Sports* 13(5): 284-292.

Asikainen, T. M., S. Miilunpalo, et al. (2002). Walking trials in postmenopausal women: effect of one vs two daily bouts on aerobic fitness. *Scand J Med Sci Sports* 12(2): 99-105.

Astrup, A. (1999). Physical activity and weight gain and fat distribution changes with menopause: current evidence and research issues. *Med Sci Sports Exerc* 31(11 Suppl): S564-567.

Barlet, J. P., V. Coxam, et al. (1994). [Animal models of post-menopausal osteoporosis]. *Reprod Nutr Dev* 34(3): 221-236.

Barsalani, R., A. Pighon, et al. (2008). Liver of ovariectomized rats is resistant to resorption of lipids. *Physiol Behav* 95(1-2): 216-221.

Barsalani, R., N.A. Chapados, J-M Lavoie. (2010). Hepatic VLDL-TG production and MTP gene expression are decreased in ovariectomized rats: effects of exercise training. Submitted to *Hormone and Metabolic Research*: (HMR-2010-02-0055).

Barzilay, J. I., L. Abraham, et al. (2001). The relation of markers of inflammation to the development of glucose disorders in the elderly: the Cardiovascular Health Study. *Diabetes* 50(10): 2384-2389.

Bastard, J. P., M. Maachi, et al. (2006). Recent advances in the relationship between obesity, inflammation, and insulin resistance. *Eur Cytokine Netw* 17(1): 4-12.

Bataller, R., P. Sancho-Bru, et al. (2003). Activated human hepatic stellate cells express the renin-angiotensin system and synthesize angiotensin II. *Gastroenterology* 125(1): 117-125.

Beckett, T., A. Tchernof, et al. (2002). Effect of ovariectomy and estradiol replacement on skeletal muscle enzyme activity in female rats. *Metabolism* 51(11): 1397-1401.

Bemben, D. A., N. L. Fetters, et al. (2000). Musculoskeletal responses to high- and low-intensity resistance training in early postmenopausal women. *Med Sci Sports Exerc* 32(11): 1949-1957.

Beral, V. (2003). Breast cancer and hormone-replacement therapy in the Million Women Study. *Lancet* 362(9382): 419-427.

Bermudez, E. A., N. Rifai, et al. (2002). Relation between markers of systemic vascular inflammation and smoking in women. *Am J Cardiol* 89(9): 1117-1119.

Bjornstrom, L. and M. Sjoberg (2005). Mechanisms of estrogen receptor signaling: convergence of genomic and nongenomic actions on target genes. *Mol Endocrinol* 19(4): 833-842.

Boden, G. (2006). Fatty acid-induced inflammation and insulin resistance in skeletal muscle and liver. *Curr Diab Rep* 6(3): 177-181.

Bolger, A. P., R. Sharma, et al. (2002). Effect of interleukin-10 on the production of tumor necrosis factor-alpha by peripheral blood mononuclear cells from patients with chronic heart failure. *Am J Cardiol* 90(4): 384-389.

Booth, F. W. and C. M. Tipton (1969). Effects of training and 17-B estradiol upon heart rates, organ weights, and ligamentous strength of female rats. *Int Z Angew Physiol* 27(3): 187-197.

Brea, A., D. Mosquera, et al. (2005). Nonalcoholic fatty liver disease is associated with carotid atherosclerosis: a case-control study. *Arterioscler Thromb Vasc Biol* 25(5): 1045-1050.

Brochu, M., M. F. Malita, et al. (2009). Resistance training does not contribute to improving the metabolic profile after a 6-month weight loss program in overweight and obese postmenopausal women. *J Clin Endocrinol Metab* 94(9): 3226-3233.

Brochu, M., R. D. Starling, et al. (2000). Visceral adipose tissue is an independent correlate of glucose disposal in older obese postmenopausal women. *J Clin Endocrinol Metab* 85(7): 2378-2384.

Bruce, K. D. and C. D. Byrne (2009). The metabolic syndrome: common origins of a multifactorial disorder. *Postgrad Med J* 85(1009): 614-621.

Bruno, S., P. Maisonneuve, et al. (2005). Incidence and risk factors for non-alcoholic steatohepatitis: prospective study of 5408 women enrolled in Italian tamoxifen chemoprevention trial. *BMJ* 330(7497): 932.

Brunt, E. M. and D. G. Tiniakos (2005). Pathological features of NASH. *Front Biosci* 10: 1475-1484.

Bruun, J. M., C. B. Nielsen, et al. (2003). Estrogen reduces pro-inflammatory cytokines in rodent adipose tissue: studies in vivo and in vitro. *Horm Metab Res* 35(3): 142-146.

Bruunsgaard, H. (2005). Physical activity and modulation of systemic low-level inflammation. *J Leukoc Biol* 78(4): 819-835.

Bruunsgaard, H. and B. K. Pedersen (2003). Age-related inflammatory cytokines and disease. *Immunol Allergy Clin North Am* 23(1): 15-39.

Bugianesi, E., N. Leone, et al. (2002). Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. *Gastroenterology* 123(1): 134-140.

Cai, D., M. Yuan, et al. (2005). Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. *Nat Med* 11(2): 183-190.

Cali, A. M., T. L. Zern, et al. (2007). Intrahepatic fat accumulation and alterations in lipoprotein composition in obese adolescents: a perfect proatherogenic state. *Diabetes Care* 30(12): 3093-3098.

Carr, M. C. (2003). The emergence of the metabolic syndrome with menopause. *J Clin Endocrinol Metab* 88(6): 2404-2411.

Carulli, L., A. Lonardo, et al. (2006). Gender, fatty liver and GGT. *Hepatology* 44(1): 278-279.

Cassidy, A. (2005). Diet and menopausal health. *Nurs Stand* 19(29): 44-52; quiz 54-45.

Chen, H. (2006). Cellular inflammatory responses: novel insights for obesity and insulin resistance. *Pharmacol Res* 53(6): 469-477.

Chitturi, S. and G. C. Farrell (2007). Fatty liver now, diabetes and heart attack later? The liver as a barometer of metabolic health. *J Gastroenterol Hepatol* 22(7): 967-969.

Choi, S. B., J. S. Jang, et al. (2005). Estrogen and exercise may enhance beta-cell function and mass via insulin receptor substrate 2 induction in ovariectomized diabetic rats. *Endocrinology* 146(11): 4786-4794.

Ciocca, D. R. and L. M. Roig (1995). Estrogen receptors in human nontarget tissues: biological and clinical implications. *Endocr Rev* 16(1): 35-62.

Clark, J. M. (2006). The epidemiology of nonalcoholic fatty liver disease in adults. *J Clin Gastroenterol* 40 Suppl 1: S5-10.

Clark, J. M., F. L. Brancati, et al. (2002). Nonalcoholic fatty liver disease. *Gastroenterology* 122(6): 1649-1657.

Colditz, G. A., W. C. Willett, et al. (1987). Menopause and the risk of coronary heart disease in women. *N Engl J Med* 316(18): 1105-1110.

Colombel, A. and B. Charbonnel (1997). Weight gain and cardiovascular risk factors in the post-menopausal women. *Hum Reprod* 12 Suppl 1: 134-145.

Cooke, P. S. and A. Naaz (2004). Role of estrogens in adipocyte development and function. *Exp Biol Med (Maywood)* 229(11): 1127-1135.

Coppack, S. W. (2001). Pro-inflammatory cytokines and adipose tissue. *Proc Nutr Soc* 60(3): 349-356.

Corbett, S. W., E. J. Wilterdink, et al. (1985). Resting oxygen consumption in over- and underfed rats with lateral hypothalamic lesions. *Physiol Behav* 35(6): 971-977.

Corriveau, P., A. Paquette, et al. (2008). Resistance training prevents liver fat accumulation in ovariectomized rats. *Maturitas* 59(3): 259-267.

Cox, K. L., V. Burke, et al. (2004). Independent and additive effects of energy restriction and exercise on glucose and insulin concentrations in sedentary overweight men. *Am J Clin Nutr* 80(2): 308-316.

Crawford, S. L., V. A. Casey, et al. (2000). A longitudinal study of weight and the menopause transition: results from the Massachusetts Women's Health Study. *Menopause* 7(2): 96-104.

D'Eon, T. M., S. C. Souza, et al. (2005). Estrogen regulation of adiposity and fuel partitioning. Evidence of genomic and non-genomic regulation of lipogenic and oxidative pathways. *J Biol Chem* 280(43): 35983-35991.

Dandona, P., A. Aljada, et al. (2005). Metabolic syndrome: a comprehensive perspective based on interactions between obesity, diabetes, and inflammation. *Circulation* 111(11): 1448-1454.

Dandona, P., R. Weinstock, et al. (1998). Tumor necrosis factor-alpha in sera of obese patients: fall with weight loss. *J Clin Endocrinol Metab* 83(8): 2907-2910.

Danesh, J., P. Whincup, et al. (2000). Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. *BMJ* 321(7255): 199-204.

Darimont, C., R. Delansorne, et al. (1997). Influence of estrogenic status on the lipolytic activity of parametrial adipose tissue in vivo: an in situ microdialysis study. *Endocrinology* 138(3): 1092-1096.

de Kleijn, M. J., Y. T. van der Schouw, et al. (2002). Endogenous estrogen exposure and cardiovascular mortality risk in postmenopausal women. *Am J Epidemiol* 155(4): 339-345.

de Lemos, E. T., F. Reis, et al. (2007). Exercise training is associated with improved levels of C-reactive protein and adiponectin in ZDF (type 2) diabetic rats. *Med Sci Monit* 13(8): BR168-174.

Deshaies, Y., A. Dagnault, et al. (1997). Interaction of corticosterone and gonadal steroids on lipid deposition in the female rat. *Am J Physiol* 273(2 Pt 1): E355-362.

Despres, J. P. (1993). Abdominal obesity as important component of insulin-resistance syndrome. *Nutrition* 9(5): 452-459.

Dinarello, C. A. (1991). Interleukin-1 and interleukin-1 antagonism. *Blood* 77(8): 1627-1652.

Dobrzyn, P., A. Dobrzyn, et al. (2004). Stearoyl-CoA desaturase 1 deficiency increases fatty acid oxidation by activating AMP-activated protein kinase in liver. *Proc Natl Acad Sci U S A* 101(17): 6409-6414.

Driggers, P. H. and J. H. Segars (2002). Estrogen action and cytoplasmic signaling pathways. Part II: the role of growth factors and phosphorylation in estrogen signaling. *Trends Endocrinol Metab* 13(10): 422-427.

Dubnov-Raz, G., A. Pines, et al. (2007). Diet and lifestyle in managing postmenopausal obesity. *Climacteric* 10 Suppl 2: 38-41.

Dulloo, A. G. and R. Calokatisa (1991). Adaptation to low calorie intake in obese mice: contribution of a metabolic component to diminished energy expenditures during and after weight loss. *Int J Obes* 15(1): 7-16.

Dulloo, A. G. and L. Girardier (1990). Adaptive changes in energy expenditure during refeeding following low-calorie intake: evidence for a specific metabolic component favoring fat storage. *Am J Clin Nutr* 52(3): 415-420.

Duncan, B. B., M. I. Schmidt, et al. (2003). Low-grade systemic inflammation and the development of type 2 diabetes: the atherosclerosis risk in communities study. *Diabetes* 52(7): 1799-1805.

Elliott, K. J., C. Sale, et al. (2002). Effects of resistance training and detraining on muscle strength and blood lipid profiles in postmenopausal women. *Br J Sports Med* 36(5): 340-344.

Eshtiaghi, R., A. Esteghamati, et al. (2010). Menopause is an independent predictor of metabolic syndrome in Iranian women. *Maturitas* 65(3): 262-266.

Esposito, K. and D. Giugliano (2004). The metabolic syndrome and inflammation: association or causation? *Nutr Metab Cardiovasc Dis* 14(5): 228-232.

Esposito, K., R. Marfella, et al. (2004). Effect of a mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. *Jama* 292(12): 1440-1446.

Evans, R. M., G. D. Barish, et al. (2004). PPARs and the complex journey to obesity. *Nat Med* 10(4): 355-361.

Evinger, A. J., 3rd and E. R. Levin (2005). Requirements for estrogen receptor alpha membrane localization and function. *Steroids* 70(5-7): 361-363.

Fabbrini, E., S. Sullivan, et al. (2010). Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. *Hepatology* 51(2): 679-689.

Faria, A. N., F. F. Ribeiro Filho, et al. (2002). Impact of visceral fat on blood pressure and insulin sensitivity in hypertensive obese women. *Obes Res* 10(12): 1203-1206.

Febbraio, M. A., N. Hiscock, et al. (2004). Interleukin-6 is a novel factor mediating glucose homeostasis during skeletal muscle contraction. *Diabetes* 53(7): 1643-1648.

Febbraio, M. A. and B. K. Pedersen (2002). Muscle-derived interleukin-6: mechanisms for activation and possible biological roles. *Faseb J* 16(11): 1335-1347.

Fisher, J. S., W. M. Kohrt, et al. (2000). Food restriction suppresses muscle growth and augments osteopenia in ovariectomized rats. *J Appl Physiol* 88(1): 265-271.

Fogelholm, M., K. Kukkonen-Harjula, et al. (2000). Effects of walking training on weight maintenance after a very-low-energy diet in premenopausal obese women: a randomized controlled trial. *Arch Intern Med* 160(14): 2177-2184.

Ford, E. S. (2002). Leukocyte count, erythrocyte sedimentation rate, and diabetes incidence in a national sample of US adults. *Am J Epidemiol* 155(1): 57-64.

Ford, E. S., W. H. Giles, et al. (2002). Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *JAMA* 287(3): 356-359.

Frank, L. L., B. E. Sorensen, et al. (2005). Effects of exercise on metabolic risk variables in overweight postmenopausal women: a randomized clinical trial. *Obes Res* 13(3): 615-625.

Frith, J. and J. L. Newton (2010). Liver disease in older women. *Maturitas* 65(3): 210-214.

Furukawa, S., T. Fujita, et al. (2004). Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest* 114(12): 1752-1761.

Gabay, C. and I. Kushner (1999). Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* 340(6): 448-454.

Gallo, D., G. F. Zannoni, et al. (2005). Characterization of the pharmacologic profile of a standardized soy extract in the ovariectomized rat model of menopause: effects on bone, uterus, and lipid profile. *Menopause* 12(5): 589-600.

Gao, Z., X. Zhang, et al. (2004). Inhibition of insulin sensitivity by free fatty acids requires activation of multiple serine kinases in 3T3-L1 adipocytes. *Mol Endocrinol* 18(8): 2024-2034.

Gauthier, M. S., K. Couturier, et al. (2003). Concurrent exercise prevents high-fat-diet-induced macrovesicular hepatic steatosis. *J Appl Physiol* 94(6): 2127-2134.

Genazzani, A. R. and M. Gambacciani (2006). Effect of climacteric transition and hormone replacement therapy on body weight and body fat distribution. *Gynecol Endocrinol* 22(3): 145-150.

Georgiadou, P. and E. Sbarouni (2009). Effect of hormone replacement therapy on inflammatory biomarkers. *Adv Clin Chem* 47: 59-93.

Gielen, S., V. Adams, et al. (2003). Anti-inflammatory effects of exercise training in the skeletal muscle of patients with chronic heart failure. *J Am Coll Cardiol* 42(5): 861-868.

Gordon, T., W. B. Kannel, et al. (1978). Menopause and coronary heart disease. The Framingham Study. *Ann Intern Med* 89(2): 157-161.

Gray, J. M. and M. R. Greenwood (1984). Effect of estrogen on lipoprotein lipase activity and cytoplasmic progestin binding sites in lean and obese Zucker rats. *Proc Soc Exp Biol Med* 175(3): 374-379.

Gray, J. M. and G. N. Wade (1981). Food intake, body weight, and adiposity in female rats: actions and interactions of progestins and antiestrogens. *Am J Physiol* 240(5): E474-481.

Greco, A. V., G. Mingrone, et al. (2002). Insulin resistance in morbid obesity: reversal with intramyocellular fat depletion. *Diabetes* 51(1): 144-151.

Green, J. S., P. R. Stanforth, et al. (2004). The effects of exercise training on abdominal visceral fat, body composition, and indicators of the metabolic syndrome in postmenopausal women with and without estrogen replacement therapy: the HERITAGE family study. *Metabolism* 53(9): 1192-1196.

Griffiths, M. A., D. H. Baker, et al. (1993). Effects of exercise training on diet-induced lipogenic enzymes and body composition in rats. *J Am Coll Nutr* 12(2): 155-161.

Grodstein, F., J. E. Manson, et al. (2000). A prospective, observational study of postmenopausal hormone therapy and primary prevention of cardiovascular disease. *Ann Intern Med* 133(12): 933-941.

Guo, S. S., C. Zeller, et al. (1999). Aging, body composition, and lifestyle: the Fels Longitudinal Study. *Am J Clin Nutr* 70(3): 405-411.

Guthrie, J. R., L. Dennerstein, et al. (2003). "Central abdominal fat and endogenous hormones during the menopausal transition." *Fertil Steril* 79(6): 1335-1340.

Hagberg, J. M., J. M. Zmuda, et al. (2000). Determinants of body composition in postmenopausal women. *J Gerontol A Biol Sci Med Sci* 55(10): M607-612.

Hagey, A. R. and M. P. Warren (2008). Role of exercise and nutrition in menopause. *Clin Obstet Gynecol* 51(3): 627-641.

Hagmar, M., A. L. Hirschberg, et al. (2005). Athlete's heart in postmenopausal former elite endurance female athletes. *Clin J Sport Med* 15(4): 257-262.

Hagymasi, K., P. Reismann, et al. (2009). [Role of the endocrine system in the pathogenesis of non-alcoholic fatty liver disease]. *Orv Hetil* 150(48): 2173-2181.

Hamilton, K. L., L. Lin, et al. (2008). Effect of ovariectomy on cardiac gene expression: inflammation and changes in SOCS gene expression. *Physiol Genomics* 32(2): 254-263.

Hamosh, M. and P. Hamosh (1975). The effect of estrogen on the lipoprotein lipase activity of rat adipose tissue. *J Clin Invest* 55(5): 1132-1135.

Hansen, D., P. Dendale, et al. (2007). The effects of exercise training on fat-mass loss in obese patients during energy intake restriction. *Sports Med* 37(1): 31-46.

Hansen, P. A., T. J. McCarthy, et al. (1996). Effects of ovariectomy and exercise training on muscle GLUT-4 content and glucose metabolism in rats. *J Appl Physiol* 80(5): 1605-1611.

Harris, T. B., L. Ferrucci, et al. (1999). Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. *Am J Med* 106(5): 506-512.

Hashem, R. M., M. F. Mahmoud, et al. (2008). Interleukin-10 to tumor necrosis factor-alpha ratio is a predictive biomarker in nonalcoholic fatty liver disease: interleukin-10 to tumor necrosis factor-alpha ratio in steatohepatitis. *Eur J Gastroenterol Hepatol* 20(10): 995-1001.

Hashimoto, T., W. S. Cook, et al. (2000). Defect in peroxisome proliferator-activated receptor alpha-inducible fatty acid oxidation determines the severity of hepatic steatosis in response to fasting. *J Biol Chem* 275(37): 28918-28928.

Hassager, C. and C. Christiansen (1989). Estrogen/gestagen therapy changes soft tissue body composition in postmenopausal women. *Metabolism* 38(7): 662-665.

Heine, P. A., J. A. Taylor, et al. (2000). Increased adipose tissue in male and female estrogen receptor-alpha knockout mice. *Proc Natl Acad Sci U S A* 97(23): 12729-12734.

Hill, J. O., S. Thacker, et al. (1988). Influence of food restriction coupled with weight cycling on carcass energy restoration during ad-libitum refeeding. *Int J Obes* 12(6): 547-555.

Hirosumi, J., G. Tuncman, et al. (2002). A central role for JNK in obesity and insulin resistance. *Nature* 420(6913): 333-336.

Hotamisligil, G. S. (2006). Inflammation and metabolic disorders. *Nature* 444(7121): 860-867.

Hotamisligil, G. S., P. Peraldi, et al. (1996). IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. *Science* 271(5249): 665-668.

Irwin, M. L., Y. Yasui, et al. (2003). Effect of exercise on total and intra-abdominal body fat in postmenopausal women: a randomized controlled trial. *JAMA* 289(3): 323-330.

Isidori, A. M., E. Giannetta, et al. (2005). Androgens, cardiovascular disease and osteoporosis. *J Endocrinol Invest* 28(10 Suppl): 73-79.

Jansen, S. C., E. H. Temme, et al. (2002). Lifetime estrogen exposure versus age at menopause as mortality predictor. *Maturitas* 43(2): 105-112.

Johnson, N. A., T. Sachinwalla, et al. (2009). Aerobic exercise training reduces hepatic and visceral lipids in obese individuals without weight loss. *Hepatology* 50(4): 1105-1112.

Jones, M. E., A. W. Thorburn, et al. (2001). Aromatase-deficient (ArKO) mice accumulate excess adipose tissue. *J Steroid Biochem Mol Biol* 79(1-5): 3-9.

Jones, M. E., A. W. Thorburn, et al. (2000). Aromatase-deficient (ArKO) mice have a phenotype of increased adiposity. *Proc Natl Acad Sci U S A* 97(23): 12735-12740.

Joshi-Barve, S., S. S. Barve, et al. (2007). Palmitic acid induces production of proinflammatory cytokine interleukin-8 from hepatocytes. *Hepatology* 46(3): 823-830.

Kaaja, R. J. (2008). Metabolic syndrome and the menopause. *Menopause Int* 14(1): 21-25.

Kadowaki, T., K. Hara, et al. (2003). Molecular mechanism of insulin resistance and obesity. *Exp Biol Med (Maywood)* 228(10): 1111-1117.

Kalani, R., S. Judge, et al. (2006). Effects of caloric restriction and exercise on age-related, chronic inflammation assessed by C-reactive protein and interleukin-6. *J Gerontol A Biol Sci Med Sci* 61(3): 211-217.

Kalu, D. N. (1991). The ovariectomized rat model of postmenopausal bone loss. *Bone Miner* 15(3): 175-191.

Kannel, W. B., L. A. Cupples, et al. (1991). Regional obesity and risk of cardiovascular disease; the Framingham Study. *J Clin Epidemiol* 44(2): 183-190.

Kasapis, C. and P. D. Thompson (2005). The effects of physical activity on serum C-reactive protein and inflammatory markers: a systematic review. *J Am Coll Cardiol* 45(10): 1563-1569.

Kaur, K., A. K. Sharma, et al. (2006). Interplay of TNF-alpha and IL-10 in regulating oxidative stress in isolated adult cardiac myocytes. *J Mol Cell Cardiol* 41(6): 1023-1030.

Keller, C., P. Keller, et al. (2004). Exercise normalises overexpression of TNF-alpha in knockout mice. *Biochem Biophys Res Commun* 321(1): 179-182.

Keller, C., A. Steensberg, et al. (2001). Transcriptional activation of the IL-6 gene in human contracting skeletal muscle: influence of muscle glycogen content. *Faseb J* 15(14): 2748-2750.

Kelly, M., C. Keller, et al. (2004). AMPK activity is diminished in tissues of IL-6 knockout mice: the effect of exercise. *Biochem Biophys Res Commun* 320(2): 449-454.

Kelly, M. J. and E. R. Levin (2001). Rapid actions of plasma membrane estrogen receptors. *Trends Endocrinol Metab* 12(4): 152-156.

Kershaw, E. E. and J. S. Flier (2004). Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab* 89(6): 2548-2556.

Kim, H. J., T. Higashimori, et al. (2004). Differential effects of interleukin-6 and -10 on skeletal muscle and liver insulin action in vivo. *Diabetes* 53(4): 1060-1067.

Kim, J. K., J. J. Fillmore, et al. (2001). Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance. *Proc Natl Acad Sci U S A* 98(13): 7522-7527.

King, D. E., P. Carek, et al. (2003). Inflammatory markers and exercise: differences related to exercise type. *Med Sci Sports Exerc* 35(4): 575-581.

Kireev, R. A., A. C. Tresguerres, et al. (2010). Hormonal regulation of pro-inflammatory and lipid peroxidation processes in liver of old ovariectomized female rats. *Biogerontology* 11(2): 229-243.

Klein, S. (2004). The case of visceral fat: argument for the defense. *J Clin Invest* 113(11): 1530-1532.

Klem, M. L., R. R. Wing, et al. (1997). A descriptive study of individuals successful at long-term maintenance of substantial weight loss. *Am J Clin Nutr* 66(2): 239-246.

Kolling, U. K., F. Hansen, et al. (2001). Leucocyte response and anti-inflammatory cytokines in community acquired pneumonia. *Thorax* 56(2): 121-125.

Kopelman, P. G. (2000). Obesity as a medical problem. *Nature* 404(6778): 635-643.

Kotronen, A., A. Seppala-Lindroos, et al. (2008). Tissue specificity of insulin resistance in humans: fat in the liver rather than muscle is associated with features of the metabolic syndrome. *Diabetologia* 51(1): 130-138.

Kotronen, A., J. Westerbacka, et al. (2007). Liver fat in the metabolic syndrome. *J Clin Endocrinol Metab* 92(9): 3490-3497.

Kotronen, A. and H. Yki-Jarvinen (2008). Fatty liver: a novel component of the metabolic syndrome. *Arterioscler Thromb Vasc Biol* 28(1): 27-38.

Kraegen, E. W., P. W. Clark, et al. (1991). Development of muscle insulin resistance after liver insulin resistance in high-fat-fed rats. *Diabetes* 40(11): 1397-1403.

Krotkiewski, M., P. Bjorntorp, et al. (1983). Impact of obesity on metabolism in men and women. Importance of regional adipose tissue distribution. *J Clin Invest* 72(3): 1150-1162.

Ksontini, R., S. L. MacKay, et al. (1998). Revisiting the role of tumor necrosis factor alpha and the response to surgical injury and inflammation. *Arch Surg* 133(5): 558-567.

Lapidus, L., C. Bengtsson, et al. (1984). Distribution of adipose tissue and risk of cardiovascular disease and death: a 12 year follow up of participants in the population study of women in Gothenburg, Sweden. *Br Med J (Clin Res Ed)* 289(6454): 1257-1261.

Latour, M. G., M. Shinoda, et al. (2001). Metabolic effects of physical training in ovariectomized and hyperestrogenic rats. *J Appl Physiol* 90(1): 235-241.

Lavoie, J. M. and M. S. Gauthier (2006). Regulation of fat metabolism in the liver: link to non-alcoholic hepatic steatosis and impact of physical exercise. *Cell Mol Life Sci* 63(12): 1393-1409.

Leite, R. D., J. Prestes, et al. (2009). Effects of ovariectomy and resistance training on lipid content in skeletal muscle, liver, and heart; fat depots; and lipid profile. *Appl Physiol Nutr Metab* 34(6): 1079-1086.

Lemieux, C., F. Picard, et al. (2003). The estrogen antagonist EM-652 and dehydroepiandrosterone prevent diet- and ovariectomy-induced obesity. *Obes Res* 11(3): 477-490.

Lemoine, S., P. Granier, et al. (2002). Effect of endurance training on oestrogen receptor alpha transcripts in rat skeletal muscle. *Acta Physiol Scand* 174(3): 283-289.

Leonidou, L., A. Mouzaki, et al. (2007). Cytokine production and hospital mortality in patients with sepsis-induced stress hyperglycemia. *J Infect* 55(4): 340-346.

Levin, B. E. and A. A. Dunn-Meynell (2000). Defense of body weight against chronic caloric restriction in obesity-prone and -resistant rats. *Am J Physiol Regul Integr Comp Physiol* 278(1): R231-237.

Levin, B. E. and A. A. Dunn-Meynell (2004). Chronic exercise lowers the defended body weight gain and adiposity in diet-induced obese rats. *Am J Physiol Regul Integr Comp Physiol* 286(4): R771-778.

Lieberman, E. H., M. D. Gerhard, et al. (1994). Estrogen improves endothelium-dependent, flow-mediated vasodilation in postmenopausal women. *Ann Intern Med* 121(12): 936-941.

Lobo, R. A. (2008). Metabolic syndrome after menopause and the role of hormones. *Maturitas* 60(1): 10-18.

Lonardo, A., C. Carani, et al. (2006). 'Endocrine NAFLD' a hormonocentric perspective of nonalcoholic fatty liver disease pathogenesis. *J Hepatol* 44(6): 1196-1207.

Loria, P., A. Lonardo, et al. (2008). Is liver fat detrimental to vessels?: intersections in the pathogenesis of NAFLD and atherosclerosis. *Clin Sci (Lond)* 115(1): 1-12.

Lovejoy, J. C., C. M. Champagne, et al. (2008). Increased visceral fat and decreased energy expenditure during the menopausal transition. *Int J Obes (Lond)* 32(6): 949-958.

Maachi, M., L. Pieroni, et al. (2004). Systemic low-grade inflammation is related to both circulating and adipose tissue TNFalpha, leptin and IL-6 levels in obese women. *Int J Obes Relat Metab Disord* 28(8): 993-997.

MacLean, P. S., J. A. Higgins, et al. (2006). Peripheral metabolic responses to prolonged weight reduction that promote rapid, efficient regain in obesity-prone rats. *Am J Physiol Regul Integr Comp Physiol* 290(6): R1577-1588.

MacLean, P. S., J. A. Higgins, et al. (2004). Enhanced metabolic efficiency contributes to weight regain after weight loss in obesity-prone rats. *Am J Physiol Regul Integr Comp Physiol* 287(6): R1306-1315.

MacLean, P. S., J. A. Higgins, et al. (2004). Metabolic adjustments with the development, treatment, and recurrence of obesity in obesity-prone rats. *Am J Physiol Regul Integr Comp Physiol* 287(2): R288-297.

Maddalozzo, G. F., J. J. Widrick, et al. (2007). The effects of hormone replacement therapy and resistance training on spine bone mineral density in early postmenopausal women. *Bone* 40(5): 1244-1251.

Maesta, N., E. A. Nahas, et al. (2007). Effects of soy protein and resistance exercise on body composition and blood lipids in postmenopausal women. *Maturitas* 56(4): 350-358.

Malavazos, A. E., G. Gobbo, et al. (2009). Lifestyle intervention and fatty liver disease: The importance of both disrupting inflammation and reducing visceral fat. *Hepatology* 51(3): 1091-1092.

Marchesini, G., M. Brizi, et al. (1999). Association of nonalcoholic fatty liver disease with insulin resistance. *Am J Med* 107(5): 450-455.

Mastorakos, G., G. Valsamakis, et al. (2010). Management of obesity in menopause: diet, exercise, pharmacotherapy and bariatric surgery. *Maturitas* 65(3): 219-224.

Maton, A., J. Hopkins, et al. (1993). *Human Biology and Health*. Englewood Cliffs, New Jersey, USA: Prentice Hall.

Matsusue, K., M. Haluzik, et al. (2003). Liver-specific disruption of PPARgamma in leptin-deficient mice improves fatty liver but aggravates diabetic phenotypes. *J Clin Invest* 111(5): 737-747.

Matthews, J. and J. A. Gustafsson (2003). Estrogen signaling: a subtle balance between ER alpha and ER beta. *Mol Interv* 3(5): 281-292.

Matthews, K. A., E. Meilahn, et al. (1989). Menopause and risk factors for coronary heart disease. *N Engl J Med* 321(10): 641-646.

Mattusch, F., B. Dufaux, et al. (2000). Reduction of the plasma concentration of C-reactive protein following nine months of endurance training. *Int J Sports Med* 21(1): 21-24.

Mayes, J. S. and G. H. Watson (2004). Direct effects of sex steroid hormones on adipose tissues and obesity. *Obes Rev* 5(4): 197-216.

McFarlin, B. K., M. G. Flynn, et al. (2006). Physical activity status, but not age, influences inflammatory biomarkers and toll-like receptor 4. *J Gerontol A Biol Sci Med Sci* 61(4): 388-393.

McKenzie, J., B. M. Fisher, et al. (2006). Effects of HRT on liver enzyme levels in women with type 2 diabetes: a randomized placebo-controlled trial. *Clin Endocrinol (Oxf)* 65(1): 40-44.

McPherson, K. (2004). Where are we now with hormone replacement therapy? *BMJ* 328(7436): 357-358.

Messier, V., A. D. Karelis, et al. (2010). Metabolically healthy but obese individuals: relationship with hepatic enzymes. *Metabolism* 59(1): 20-24.

Meza-Munoz, D. E., M. E. Fajardo, et al. (2006). Factors associated with estrogen receptors-alpha (ER-alpha) and -beta (ER-beta) and progesterone receptor abundance in obese and non obese pre- and post-menopausal women. *Steroids* 71(6): 498-503.

Milewicz, A., M. Demissie, et al. (2003). Influence of dietary and genetic factors on metabolic status in obese and lean postmenopausal women. *Gynecol Endocrinol* 17(4): 333-338.

Miller, V. M. and S. P. Duckles (2008). Vascular actions of estrogens: functional implications. *Pharmacol Rev* 60(2): 210-241.

Mishima, Y., A. Kuyama, et al. (2001). Relationship between serum tumor necrosis factor-alpha and insulin resistance in obese men with Type 2 diabetes mellitus. *Diabetes Res Clin Pract* 52(2): 119-123.

Misso, M. L., Y. Murata, et al. (2003). Cellular and molecular characterization of the adipose phenotype of the aromatase-deficient mouse. *Endocrinology* 144(4): 1474-1480.

Mohamed-Ali, V., S. Goodrick, et al. (1997). Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. *J Clin Endocrinol Metab* 82(12): 4196-4200.

Mokdad, A. H., M. K. Serdula, et al. (1999). The spread of the obesity epidemic in the United States, 1991-1998. *JAMA* 282(16): 1519-1522.

Moldoveanu, A. I., R. J. Shephard, et al. (2001). The cytokine response to physical activity and training. *Sports Med* 31(2): 115-144.

Musso, G., R. Gambino, et al. (2008). Should nonalcoholic fatty liver disease be included in the definition of metabolic syndrome? A cross-sectional comparison with Adult Treatment Panel III criteria in nonobese nondiabetic subjects. *Diabetes Care* 31(3): 562-568.

Musso, G., R. Gambino, et al. (2009). Recent insights into hepatic lipid metabolism in non-alcoholic fatty liver disease (NAFLD). *Prog Lipid Res* 48(1): 1-26.

Musso, G., R. Gambino, et al. (2003). Dietary habits and their relations to insulin resistance and postprandial lipemia in nonalcoholic steatohepatitis. *Hepatology* 37(4): 909-916.

Na, X. L., J. Ezaki, et al. (2008). Isoflavone regulates lipid metabolism via expression of related genes in OVX rats fed on a high-fat diet. *Biomed Environ Sci* 21(5): 357-364.

Nassis, G. P., K. Papantakou, et al. (2005). Aerobic exercise training improves insulin sensitivity without changes in body weight, body fat, adiponectin, and inflammatory markers in overweight and obese girls. *Metabolism* 54(11): 1472-1479.

Nemoto, Y., K. Toda, et al. (2000). Altered expression of fatty acid-metabolizing enzymes in aromatase-deficient mice. *J Clin Invest* 105(12): 1819-1825.

Nguyen-Duy, T. B., M. Z. Nichaman, et al. (2003). Visceral fat and liver fat are independent predictors of metabolic risk factors in men. *Am J Physiol Endocrinol Metab* 284(6): E1065-1071.

Nicklas, B. J., E. M. Rogus, et al. (1997). Exercise blunts declines in lipolysis and fat oxidation after dietary-induced weight loss in obese older women. *Am J Physiol* 273(1 Pt 1): E149-155.

Oberbach, A., A. Tonjes, et al. (2006). Effect of a 4 week physical training program on plasma concentrations of inflammatory markers in patients with abnormal glucose tolerance. *Eur J Endocrinol* 154(4): 577-585.

Ohlsson, C., N. Hellberg, et al. (2000). Obesity and disturbed lipoprotein profile in estrogen receptor-alpha-deficient male mice. *Biochem Biophys Res Commun* 278(3): 640-645.

Okura, T., Y. Nakata, et al. (2004). Regional body composition changes exhibit opposing effects on coronary heart disease risk factors. *Arterioscler Thromb Vasc Biol* 24(5): 923-929.

Orsatti, F. L., E. A. Nahas, et al. (2008). Plasma hormones, muscle mass and strength in resistance-trained postmenopausal women. *Maturitas* 59(4): 394-404.

Ostrowski, K., T. Rohde, et al. (1999). Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. *J Physiol* 515 (Pt 1): 287-291.

Ozcan, U., Q. Cao, et al. (2004). Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science* 306(5695): 457-461.

Pallottini, V., P. Bulzomi, et al. (2008). Estrogen regulation of adipose tissue functions: involvement of estrogen receptor isoforms. *Infect Disord Drug Targets* 8(1): 52-60.

Panotopoulos, G., J. Raison, et al. (1997). Weight gain at the time of menopause. *Hum Reprod* 12 Suppl 1: 126-133.

Paquette, A., N. A. Chapados, et al. (2009). Fatty acid oxidation is decreased in the liver of ovariectomized rats. *Horm Metab Res* 41(7): 511-515.

Paquette, A., M. Shinoda, et al. (2007). Time course of liver lipid infiltration in ovariectomized rats: impact of a high-fat diet. *Maturitas* 58(2): 182-190.

Paquette, A., D. Wang, et al. (2007). Specific adaptations of estrogen receptor alpha and beta transcripts in liver and heart after endurance training in rats. *Mol Cell Biochem* 306(1-2): 179-187.

Paquette, A., D. Wang, et al. (2008). Effects of ovariectomy on PPAR alpha, SREBP-1c, and SCD-1 gene expression in the rat liver. *Menopause* 15(6): 1169-1175.

Park, H., V. K. Kaushik, et al. (2002). Coordinate regulation of malonyl-CoA decarboxylase, sn-glycerol-3-phosphate acyltransferase, and acetyl-CoA carboxylase by AMP-activated protein kinase in rat tissues in response to exercise. *J Biol Chem* 277(36): 32571-32577.

Park, S. H., W. K. Jeon, et al. (2006). Prevalence and risk factors of non-alcoholic fatty liver disease among Korean adults. *J Gastroenterol Hepatol* 21(1 Pt 1): 138-143.

Park, Y. W., S. Zhu, et al. (2003). The metabolic syndrome: prevalence and associated risk factor findings in the US population from the Third National Health and Nutrition Examination Survey, 1988-1994. *Arch Intern Med* 163(4): 427-436.

Patterson, C. M., A. A. Dunn-Meynell, et al. (2008). Three weeks of early-onset exercise prolongs obesity resistance in DIO rats after exercise cessation. *Am J Physiol Regul Integr Comp Physiol* 294(2): R290-301.

Pavon de Paz, I., C. Alameda Hernando, et al. (2006). [Obesity and menopause]. *Nutr Hosp* 21(6): 633-637.

Pedersen, B. K. (2009). The diseasome of physical inactivity--and the role of myokines in muscle--fat cross talk. *J Physiol* 587(Pt 23): 5559-5568.

Pedersen, B. K., A. Steensberg, et al. (2003). Searching for the exercise factor: is IL-6 a candidate? *J Muscle Res Cell Motil* 24(2-3): 113-119.

Pedersen, S. B., J. M. Bruun, et al. (2001). Regulation of UCP1, UCP2, and UCP3 mRNA expression in brown adipose tissue, white adipose tissue, and skeletal muscle in rats by estrogen. *Biochem Biophys Res Commun* 288(1): 191-197.

Percegoni, N., A. C. Ferreira, et al. (2009). Profile of serum IL-1beta and IL-10 shortly after ovariectomy and estradiol replacement in rats. *Horm Metab Res* 41(1): 50-54.

Petersen, A. M. and B. K. Pedersen (2005). The anti-inflammatory effect of exercise. *J Appl Physiol* 98(4): 1154-1162.

Petersen, K. F., S. Dufour, et al. (2005). Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate weight reduction in patients with type 2 diabetes. *Diabetes* 54(3): 603-608.

Petta, S., C. Muratore, et al. (2009). Non-alcoholic fatty liver disease pathogenesis: the present and the future. *Dig Liver Dis* 41(9): 615-625.

Pfeilschifter, J., R. Koditz, et al. (2002). Changes in proinflammatory cytokine activity after menopause. *Endocr Rev* 23(1): 90-119.

Picard, F., Y. Deshaies, et al. (2000). Effects of the estrogen antagonist EM-652.HCl on energy balance and lipid metabolism in ovariectomized rats. *Int J Obes Relat Metab Disord* 24(7): 830-840.

Pines, A. and E. M. Berry (2007). Exercise in the menopause - an update. *Climacteric* 10 Suppl 2: 42-46.

Psaty, B. M., S. R. Heckbert, et al. (1994). The risk of myocardial infarction associated with the combined use of estrogens and progestins in postmenopausal women. *Arch Intern Med* 154(12): 1333-1339.

Rainville, S. and P. Vaccaro (1984). The effects of menopause and training on serum lipids. *Int J Sports Med* 5(3): 137-141.

Ready, A. E., D. T. Drinkwater, et al. (1995). Walking program reduces elevated cholesterol in women postmenopause. *Can J Cardiol* 11(10): 905-912.

Ready, A. E., B. Naimark, et al. (1996). Influence of walking volume on health benefits in women post-menopause. *Med Sci Sports Exerc* 28(9): 1097-1105.

Reddy, J. K. and M. S. Rao (2006). Lipid metabolism and liver inflammation. II. Fatty liver disease and fatty acid oxidation. *Am J Physiol Gastrointest Liver Physiol* 290(5): G852-858.

Renno, A. C., A. R. Silveira Gomes, et al. (2007). Effects of a progressive loading exercise program on the bone and skeletal muscle properties of female osteopenic rats. *Exp Gerontol* 42(6): 517-522.

Revankar, C. M., D. F. Cimino, et al. (2005). A transmembrane intracellular estrogen receptor mediates rapid cell signaling. *Science* 307(5715): 1625-1630.

Richard, D. (1986). Effects of ovarian hormones on energy balance and brown adipose tissue thermogenesis. *Am J Physiol* 250(2 Pt 2): R245-249.

Richard, D., L. Rochon, et al. (1987). Effects of exercise training on energy balance of ovariectomized rats. *Am J Physiol* 253(5 Pt 2): R740-745.

Ridker, P. M., M. Cushman, et al. (1997). Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med* 336(14): 973-979.

Ridker, P. M., C. H. Hennekens, et al. (2000). C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med* 342(12): 836-843.

Ridker, P. M., N. Rifai, et al. (2000). Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation* 101(15): 1767-1772.

Rocca, W. A., B. R. Grossardt, et al. (2006). Survival patterns after oophorectomy in premenopausal women: a population-based cohort study. *Lancet Oncol* 7(10): 821-828.

Rogers, A. and R. Eastell (2001). The effect of 17beta-estradiol on production of cytokines in cultures of peripheral blood. *Bone* 29(1): 30-34.

Rogers, N. H., J. W. Perfield, 2nd, et al. (2009). Reduced energy expenditure and increased inflammation are early events in the development of ovariectomy-induced obesity. *Endocrinology* 150(5): 2161-2168.

Ronti, T., G. Lupattelli, et al. (2006). The endocrine function of adipose tissue: an update. *Clin Endocrinol (Oxf)* 64(4): 355-365.

Ropero, A. B., E. Fuentes, et al. (1999). Non-genomic actions of 17beta-oestradiol in mouse pancreatic beta-cells are mediated by a cGMP-dependent protein kinase. *J Physiol* 521 Pt 2: 397-407.

Rosano, G. M., C. Vitale, et al. (2007). Menopause and cardiovascular disease: the evidence. *Climacteric* 10 Suppl 1: 19-24.

Rosenberg, L., C. H. Hennekens, et al. (1981). Early menopause and the risk of myocardial infarction. *Am J Obstet Gynecol* 139(1): 47-51.

Ross, R. (1999). Atherosclerosis--an inflammatory disease. *N Engl J Med* 340(2): 115-126.

Ross, R., D. Dagnone, et al. (2000). Reduction in obesity and related comorbid conditions after diet-induced weight loss or exercise-induced weight loss in men. A randomized, controlled trial. *Ann Intern Med* 133(2): 92-103.

Ross, R., I. Janssen, et al. (2004). Exercise-induced reduction in obesity and insulin resistance in women: a randomized controlled trial. *Obes Res* 12(5): 789-798.

Rossouw, J. E., G. L. Anderson, et al. (2002). Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *JAMA* 288(3): 321-333.

Roy, E. J. and G. N. Wade (1975). Role of estrogens in androgen-induced spontaneous activity in male rats. *J Comp Physiol Psychol* 89(6): 573-579.

Ryan, A. S., R. E. Pratley, et al. (1995). Resistive training increases fat-free mass and maintains RMR despite weight loss in postmenopausal women. *J Appl Physiol* 79(3): 818-823.

Ryan, A. S., R. E. Pratley, et al. (2000). Changes in plasma leptin and insulin action with resistive training in postmenopausal women. *Int J Obes Relat Metab Disord* 24(1): 27-32.

Ryan, A. S., R. E. Pratley, et al. (1996). Resistive training increases insulin action in postmenopausal women. *J Gerontol A Biol Sci Med Sci* 51(5): M199-205.

Saengsirisuwan, V., S. Pongseeda, et al. (2009). Modulation of insulin resistance in ovariectomized rats by endurance exercise training and estrogen replacement. *Metabolism* 58(1): 38-47.

Saghizadeh, M., J. M. Ong, et al. (1996). The expression of TNF alpha by human muscle. Relationship to insulin resistance. *J Clin Invest* 97(4): 1111-1116.

Salt, W. B., 2nd (2004). Nonalcoholic fatty liver disease (NAFLD): a comprehensive review. *J Insur Med* 36(1): 27-41.

Saltiki, K., C. Doukas, et al. (2006). Severity of cardiovascular disease in women: relation with exposure to endogenous estrogen. *Maturitas* 55(1): 51-57.

Samuel, V. T., Z. X. Liu, et al. (2004). Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. *J Biol Chem* 279(31): 32345-32353.

Santidrian, S. and J. R. Thompson (1981). Effect of estradiol benzoate on the rate of myofibrillar protein degradation in growing ovariectomized female rats. *Arch Farmacol Toxicol* 7(2): 215-222.

Sanyal, A. J., C. Campbell-Sargent, et al. (2001). Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology* 120(5): 1183-1192.

Scheen, A. J. and F. H. Luyckx (2002). Obesity and liver disease. *Best Pract Res Clin Endocrinol Metab* 16(4): 703-716.

Schenk, S., M. Saberi, et al. (2008). Insulin sensitivity: modulation by nutrients and inflammation. *J Clin Invest* 118(9): 2992-3002.

Schottelius, A. J., M. W. Mayo, et al. (1999). Interleukin-10 signaling blocks inhibitor of kappaB kinase activity and nuclear factor kappaB DNA binding. *J Biol Chem* 274(45): 31868-31874.

Seed, M. (2002). The choice of hormone replacement therapy or statin therapy in the treatment of hyperlipidemic postmenopausal women. *Atheroscler Suppl* 3(1): 53-63.

Segars, J. H. and P. H. Driggers (2002). Estrogen action and cytoplasmic signaling cascades. Part I: membrane-associated signaling complexes. *Trends Endocrinol Metab* 13(8): 349-354.

Seidlova-Wuttke, D., O. Hesse, et al. (2003). Evidence for selective estrogen receptor modulator activity in a black cohosh (Cimicifuga racemosa) extract: comparison with estradiol-17beta. *Eur J Endocrinol* 149(4): 351-362.

Seidlova-Wuttke, D., H. Jarry, et al. (2003). Pharmacology of Cimicifuga racemosa extract BNO 1055 in rats: bone, fat and uterus. *Maturitas* 44 Suppl 1: S39-50.

Shaw, K., H. Gennat, et al. (2006). Exercise for overweight or obesity. *Cochrane Database Syst Rev*(4): CD003817.

Shi, H., M. V. Kokoeva, et al. (2006). TLR4 links innate immunity and fatty acid-induced insulin resistance. *J Clin Invest* 116(11): 3015-3025.

Shimizu, I. (2003). Impact of oestrogens on the progression of liver disease. *Liver Int* 23(1): 63-69.

Shinoda, M., M. G. Latour, et al. (2002). Effects of physical training on body composition and organ weights in ovariectomized and hyperestrogenic rats. *Int J Obes Relat Metab Disord* 26(3): 335-343.

Shoelson, S. E., L. Herrero, et al. (2007). Obesity, inflammation, and insulin resistance. *Gastroenterology* 132(6): 2169-2180.

Shoelson, S. E., J. Lee, et al. (2006). Inflammation and insulin resistance. *J Clin Invest* 116(7): 1793-1801.

Sidney, S., D. B. Petitti, et al. (1997). Myocardial infarction and the use of estrogen and estrogen-progestogen in postmenopausal women. *Ann Intern Med* 127(7): 501-508.

Sieminska, L., A. Cichon-Lenart, et al. (2006). [Sex hormones and adipocytokines in postmenopausal women]. *Pol Merkur Lekarski* 20(120): 727-730.

Simkin-Silverman, L. R. and R. R. Wing (2000). Weight gain during menopause. Is it inevitable or can it be prevented? *Postgrad Med* 108(3): 47-50, 53-46.

Simkin-Silverman, L. R., R. R. Wing, et al. (2003). Lifestyle intervention can prevent weight gain during menopause: results from a 5-year randomized clinical trial. *Ann Behav Med* 26(3): 212-220.

Simoncig-Netjasov, A., S. Vujovic, et al. (2008). [Gaining weight and components of metabolic syndrome in the period of menopause]. *Srp Arh Celok Lek* 136(9-10): 505-513.

Simpson, E., M. Jones, et al. (2005). Estrogen, a fundamental player in energy homeostasis. *J Steroid Biochem Mol Biol* 95(1-5): 3-8.

Sjostrom, L., U. Smith, et al. (1972). Cellularity in different regions of adipose tissue in young men and women. *Metabolism* 21(12): 1143-1153.

Sowers, M., H. Zheng, et al. (2007). Changes in body composition in women over six years at midlife: ovarian and chronological aging. *J Clin Endocrinol Metab* 92(3): 895-901.

Stefan, N., K. Kantartzis, et al. (2008). Identification and characterization of metabolically benign obesity in humans. *Arch Intern Med* 168(15): 1609-1616.

Stein, L. L., M. H. Dong, et al. (2009). Insulin sensitizers in nonalcoholic fatty liver disease and steatohepatitis: Current status. *Adv Ther* 26(10): 893-907.

Sternfeld, B., H. Wang, et al. (2004). Physical activity and changes in weight and waist circumference in midlife women: findings from the Study of Women's Health Across the Nation. *Am J Epidemiol* 160(9): 912-922.

Stevenson, E. T., K. P. Davy, et al. (1995). Hemostatic, metabolic, and androgenic risk factors for coronary heart disease in physically active and less active postmenopausal women. *Arterioscler Thromb Vasc Biol* 15(5): 669-677.

Summers, S. A. (2006). Ceramides in insulin resistance and lipotoxicity. *Prog Lipid Res* 45(1): 42-72.

Surmi, B. K. and A. H. Hasty (2010). The role of chemokines in recruitment of immune cells to the artery wall and adipose tissue. *Vascul Pharmacol* 52(1-2): 27-36.

Suzuki, A. and M. F. Abdelmalek (2009). Nonalcoholic fatty liver disease in women. *Womens Health (Lond Engl)* 5(2): 191-203.

Tarantino, G., G. Pizza, et al. (2009). Hepatic steatosis in overweight/obese females: new screening method for those at risk. *World J Gastroenterol* 15(45): 5693-5699.

Targher, G., L. Bertolini, et al. (2007). Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. *Diabetes Care* 30(5): 1212-1218.

Targher, G., L. Bertolini, et al. (2006). Relations between carotid artery wall thickness and liver histology in subjects with nonalcoholic fatty liver disease. *Diabetes Care* 29(6): 1325-1330.

Targher, G., F. Marra, et al. (2008). Increased risk of cardiovascular disease in non-alcoholic fatty liver disease: causal effect or epiphenomenon? *Diabetologia* 51(11): 1947-1953.

Tchernof, A., J. Calles-Escandon, et al. (1998). Menopause, central body fatness, and insulin resistance: effects of hormone-replacement therapy. *Coron Artery Dis* 9(8): 503-511.

Thamer, C., J. Machann, et al. (2007). High visceral fat mass and high liver fat are associated with resistance to lifestyle intervention. *Obesity (Silver Spring)* 15(2): 531-538.

Thomas, E. L., G. Hamilton, et al. (2005). Hepatic triglyceride content and its relation to body adiposity: a magnetic resonance imaging and proton magnetic resonance spectroscopy study. *Gut* 54(1): 122-127.

Tilg, H., C. A. Dinarello, et al. (1997). IL-6 and APPs: anti-inflammatory and immunosuppressive mediators. *Immunol Today* 18(9): 428-432.

Tilg, H. and G. S. Hotamisligil (2006). Nonalcoholic fatty liver disease: Cytokine-adipokine interplay and regulation of insulin resistance. *Gastroenterology* 131(3): 934-945.

Tilg, H. and A. R. Moschen (2008). Insulin resistance, inflammation, and non-alcoholic fatty liver disease. *Trends Endocrinol Metab* 19(10): 371-379.

Tisi, P. V., M. Hulse, et al. (1997). Exercise training for intermittent claudication: does it adversely affect biochemical markers of the exercise-induced inflammatory response? *Eur J Vasc Endovasc Surg* 14(5): 344-350.

Tolman, K. G., V. Fonseca, et al. (2007). Spectrum of liver disease in type 2 diabetes and management of patients with diabetes and liver disease. *Diabetes Care* 30(3): 734-743.

Tracy, R. P., R. N. Lemaitre, et al. (1997). Relationship of C-reactive protein to risk of cardiovascular disease in the elderly. Results from the Cardiovascular Health Study and the Rural Health Promotion Project. *Arterioscler Thromb Vasc Biol* 17(6): 1121-1127.

Trayhurn, P. and I. S. Wood (2005). Signalling role of adipose tissue: adipokines and inflammation in obesity. *Biochem Soc Trans* 33(Pt 5): 1078-1081.

Ueki, K., T. Kondo, et al. (2004). Suppressor of cytokine signaling 1 (SOCS-1) and SOCS-3 cause insulin resistance through inhibition of tyrosine phosphorylation of insulin receptor substrate proteins by discrete mechanisms. *Mol Cell Biol* 24(12): 5434-5446.

van der Poll, T., S. M. Coyle, et al. (1996). Epinephrine inhibits tumor necrosis factor-alpha and potentiates interleukin 10 production during human endotoxemia. *J Clin Invest* 97(3): 713-719.

Villanova, N., S. Moscatiello, et al. (2005). Endothelial dysfunction and cardiovascular risk profile in nonalcoholic fatty liver disease. *Hepatology* 42(2): 473-480.

Villareal, D. T., C. M. Apovian, et al. (2005). Obesity in older adults: technical review and position statement of the American Society for Nutrition and NAASO, The Obesity Society. *Obes Res* 13(11): 1849-1863.

Volanakis, J. E. (2001). Human C-reactive protein: expression, structure, and function. *Mol Immunol* 38(2-3): 189-197.

Volzke, H., S. Schwarz, et al. (2007). Menopausal status and hepatic steatosis in a general female population. *Gut* 56(4): 594-595.

Vural, P., C. Akgul, et al. (2006). Effects of hormone replacement therapy on plasma pro-inflammatory and anti-inflammatory cytokines and some bone turnover markers in postmenopausal women. *Pharmacol Res* 54(4): 298-302.

Wadden, T. A., R. A. Vogt, et al. (1998). Exercise and the maintenance of weight loss: 1-year follow-up of a controlled clinical trial. *J Consult Clin Psychol* 66(2): 429-433.

Wade, G. N. and J. M. Gray (1978). Cytoplasmic 17 beta-[3H]estradiol binding in rat adipose tissues. *Endocrinology* 103(5): 1695-1701.

Wang, J. F., Y. X. Guo, et al. (2004). Effects of Radix Puerariae flavones on liver lipid metabolism in ovariectomized rats. *World J Gastroenterol* 10(13): 1967-1970.

Wegge, J. K., C. K. Roberts, et al. (2004). Effect of diet and exercise intervention on inflammatory and adhesion molecules in postmenopausal women on hormone replacement therapy and at risk for coronary artery disease. *Metabolism* 53(3): 377-381.

Weisberg, S. P., D. McCann, et al. (2003). Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 112(12): 1796-1808.

Welty, F. K. (2001). Cardiovascular disease and dyslipidemia in women. *Arch Intern Med* 161(4): 514-522.

Wenger, N. K., L. Speroff, et al. (1993). Cardiovascular health and disease in women. *N Engl J Med* 329(4): 247-256.

Wieckowska, A., B. G. Papouchado, et al. (2008). Increased hepatic and circulating interleukin-6 levels in human nonalcoholic steatohepatitis. *Am J Gastroenterol* 103(6): 1372-1379.

Wiik, A., T. Gustafsson, et al. (2005). Expression of oestrogen receptor alpha and beta is higher in skeletal muscle of highly endurance-trained than of moderately active men. *Acta Physiol Scand* 184(2): 105-112.

Wilfred de Alwis, N. M. and C. P. Day (2007). Genetics of alcoholic liver disease and nonalcoholic fatty liver disease. *Semin Liver Dis* 27(1): 44-54.

Williams, F. B., L. C. Sander, et al. (2006). Development and evaluation of methods for determination of naphthodianthrones and flavonoids in St. John's wort. *J Chromatogr A* 1115(1-2): 93-102.

Wingo, P. A., E. E. Calle, et al. (2000). How does breast cancer mortality compare with that of other cancers and selected cardiovascular diseases at different ages in U.S. women? *J Womens Health Gend Based Med* 9(9): 999-1006.

Winkler, G., F. Salamon, et al. (1998). Elevated serum tumor necrosis factor-alpha concentrations and bioactivity in Type 2 diabetics and patients with android type obesity. *Diabetes Res Clin Pract* 42(3): 169-174.

Wronski, T. J., P. A. Schenck, et al. (1987). Effect of body weight on osteopenia in ovariectomized rats. *Calcif Tissue Int* 40(3): 155-159.

Xu, H., G. T. Barnes, et al. (2003). Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* 112(12): 1821-1830.

Yakar, S., N. P. Nunez, et al. (2006). Increased tumor growth in mice with diet-induced obesity: impact of ovarian hormones. *Endocrinology* 147(12): 5826-5834.

Yeh, S. S., A. Hafner, et al. (2004). Risk factors relating blood markers of inflammation and nutritional status to survival in cachectic geriatric patients in a randomized clinical trial. *J Am Geriatr Soc* 52(10): 1708-1712.

Zanesco, A. and P. R. Zaros (2009). [Physical exercise and menopause]. *Rev Bras Ginecol Obstet* 31(5): 254-261.

Zoth, N., C. Weigt, et al. (2010). Physical activity and estrogen treatment reduce visceral body fat and serum levels of leptin in an additive manner in a diet induced animal model of obesity. *J Steroid Biochem Mol Biol*.