

**MENOPAUSE TRANSITION AND POSTMENOPAUSAL PERIOD:
RELATIONSHIP WITH INFLAMMATORY MARKERS, PHYSICAL
ACTIVITY ENERGY EXPENDITURE AND BONE MINERAL DENSITY
IN HEALTHY WOMEN**

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Thesis submitted to the Faculty of Graduate and Postdoctoral Studies
in partial fulfillment of the requirements for
the Doctorate in Philosophy in Human Kinetics

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ABSTRACT

Menopause transition is usually associated with changes in body composition and a decrease in physical activity energy expenditure. Adipose tissue, especially visceral fat, is an important source of inflammatory markers, which contributes to the development of a pro-inflammatory state. Conversely, high levels of physical activity and exercise have an anti-inflammatory effect. One-hundred and two healthy premenopausal women participated in a 5-year longitudinal observational study (MONET: Montreal Ottawa New Emerging Team). The present secondary analyses were performed on 58 participants between the ages of 47 and 54 years with a full set of data. The aim of study was to investigate the impact of menopause transition and physical activity on inflammatory markers. The major finding of the first of 3 studies was that menopausal transition is accompanied by an increase in inflammatory markers, namely ferritin, IL-8, and sTNFR 1 and 2. The increase in IL-8 and sTNFR2 with menopause could be explained, in part, by changes in fat mass and peripheral fat, respectively.

During and after menopause, significant bone loss occurs in women due to reduced estrogen production. Estrogen reduction favors bone resorption by regulating the production and activity of inflammatory markers. Therefore we further investigated the association between inflammatory markers and bone mineral density in premenopausal women transitioning to menopause (paper 2). Our results showed no significant association between change in inflammatory markers and change in bone mineral density in women transitioning to menopause. However, in premenopausal women hs-CRP was negatively associated with total, lumbar spine and femoral neck bone mineral density and along with weight and cardiorespiratory fitness may play a role in bone mineral density variation. Baseline level of hs-CRP, Hp, IL-6 and femoral neck bone mineral density along with percent change in physical activity energy expenditure and

menopausal status partly explained the individual variation of bone mineral density losses in women transitioning to menopause. Finally, we investigated time spent in the postmenopausal years and the influence of the duration of the postmenopause status on body composition and cardiometabolic risk factors. We indicated that postmenopausal years and years since menopause is associated with decrease in blood glucose and increase in waist circumference, percent fat mass, total cholesterol, and high density lipoprotein. Inflammatory markers including ApoB, ferritin, adiponectin, sCD14 were higher during years after final menstrual period while sTNFR1 and sTNFR2 were higher during the menopause transition and early postmenopausal years.

ACKNOWLEDGEMENT

First and foremost I would like to express my sincere and deepest gratitude to my thesis supervisor, Dr. Denis Prud'homme for the continuous guidance he gave me throughout my Ph.D study and research. With his patience, immense knowledge he has directed my development as a researcher. I am truly grateful for his enthusiasm and support in every aspect over the years. Without him this would have never been possible. I would also, like to extend my warmest thanks to the MONET research team members: Dr. Eric Doucet, Dr. Remi Rabasa-Lhoret, Dr. Jean Marc Lavoie. I am grateful for having a supportive thesis committee, Dr. Glen Kenny and Dr. Eric Doucet, their insightful comments and advice pushed me to widen my research from various perspectives. My sincere thanks also go to Dr Jean-Philippe Bastard, Dr Soraya Fellahi and Dr Jean-Francois Mauger for blood analysis measurements and their comments and review as well as being part of the journey. I would like to extend my warmest thanks to my dear colleague Dr Joseph Abdounour for his great assistance during my studies. I would also like to thank Mrs Ann Benianto, for having spent numerous hours helping out in all possible ways to collect participant's blood samples. I am grateful to Dr Eva Guérin for her time to review my thesis. Finally, I would like to thank my family who've been supportive during my studies and who've always believed in me. To my love Farid, who inspired me and provided constant encouragement during my PhD studies. This could not have been possible without you.

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LIST OF ABBREVIATIONS

APO-B	Apolipoprotein B
BMI	Body Mass Index
CRP	C-reactive protein
CT	Computed Tomography
DEXA	Dual-Energy X-ray Absorptiometry
FFA	Free Fatty Acid
HDL-C	High-Density-Lipoproteins Cholesterol
HOMA-IR	Insulin Resistance Homeostasis Model Assessment
IFN γ	Interferon gamma
IL-6	Interleukin 6
LDL-C	Low-Density-Lipoproteins Cholesterol
PAI-1	Plasminogen activator inhibitor-1
SAA	Serum Amyloid A
sTNFR	Soluble tumor necrosis factor receptor
TC	Total Cholesterol
TG	Triglycerides
TNF- α	Tumour necrosis factor
VO ₂ peak	Peak oxygen consumption in mlO ₂ ·kg ⁻¹ ·min ⁻¹

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CHAPTER 1

1. Introduction

The increased prevalence of risk factors for cardiovascular diseases in aging women has raised concerns among health researchers, especially in regards to body composition, body fat distribution and cardiometabolic changes that are associated with the menopausal transition and postmenopausal period.^{1,2} Among the many cardiometabolic changes observed during these periods, alteration of the inflammation status has received growing interest from the scientific community.³ In particular, chronic inflammation, which is associated with aging, has been linked with the development of obesity.¹ Chronic inflammation is also related to a variety of chronic diseases including cardiovascular diseases, type 2 diabetes, osteoporosis, and cancer.⁴ In addition, body composition and body fat distribution have been shown to change during the menopause transition. In fact, the transition from pre- to post-menopause is associated with increased fat mass and central fat, especially the accumulation of visceral abdominal fat.^{2,4} However, fat cell (adipocytes) are no longer considered to serve solely as passive storage units of fat. Rather, they are thought to be active contributors to women's cardiometabolic profiles, including the inflammatory status, and their overall health. There is mounting evidence that adipocytes act as an endocrine organ, secreting a variety of cytokines which could lead to a chronic inflammation status.⁵ Therefore the increase in body fat mass, especially visceral fat, observed across the menopausal transition may favor the development of a pro-inflammatory adipokine profile.⁶

Menopause is also associated with a shift to a more sedentary lifestyle⁷ and it has been shown that lower physical activity and cardiorespiratory fitness are related to higher levels of

inflammatory markers [i.e., C-reactive protein (CRP), fibrinogen and tissue plasminogen activator (PAI-1)].⁸ In addition, there is evidence that hormonal changes that occur during the menopausal transition, such as a progressive decline in ovarian estrogen secretion and an elevation in gonadotropin levels, can affect cytokine production.⁹ It has also been shown that estrogen regulates bone remodeling by modulating the production of cytokines.⁹ Hence, the increase in cytokine concentrations during menopausal transition is involved in the pathogenesis, development and progression of metabolic syndrome, atherosclerosis, osteoporosis and many other diseases that can affect postmenopausal women's health.⁹

It would therefore be important to further investigate the influence of the menopause transition and the effect of post-menopause-associated changes in body composition, body fat distribution and cardiometabolic parameters on inflammatory markers. It would also be relevant to determine the influence of physical activity and cardiorespiratory fitness on levels of inflammatory markers in women during the menopausal transition and during the post-menopausal period. This research program contributes to a better documentation and understanding of the factors associated with the development of a chronic inflammation status and its impact on the cardiometabolic risk factors and bone mineral density in women during the menopause transition and post-menopausal period. The results from the proposed studies may lead to improved identification of preventive interventions for women going through menopause transition and postmenopausal years.

2. Literature Review

2.1 Inflammation

Inflammation plays an important role in the development of many chronic diseases such as cardiovascular diseases, type 2 diabetes, and cancer (figure 1) and it can be classified as either acute or chronic.⁴ Obesity is one example of a sub-chronic inflammatory status. Markers of inflammation can be beneficial in diagnosing and monitoring many diseases, for instance atherosclerosis.¹⁰ Markers of inflammation can be classified into three groups: cytokines, acute phase proteins and adhesion molecules.¹¹

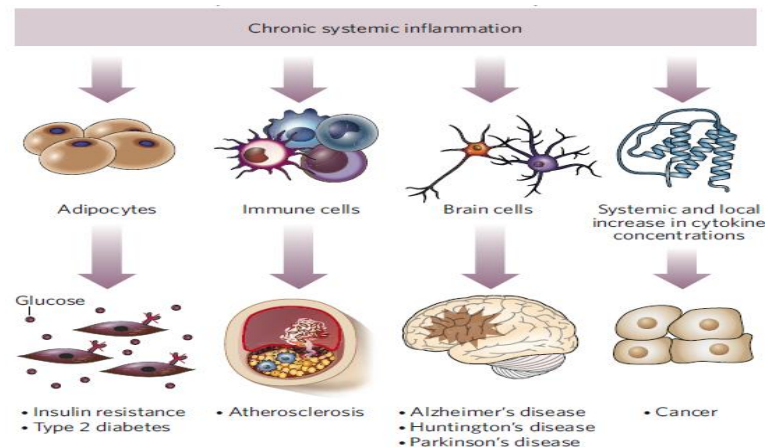


Figure 1. Inflammation and Chronic Diseases¹²

A major function of cytokines is to mediate the interactions between immune and inflammatory systems. Cytokines include interleukins, interferons, lymphokines, and tumour necrosis factor alpha (TNF- α). Cytokines are produced by multiple sources (mostly by macrophages and monocytes), and have multiple target functions. The cytokines that are involved in inflammatory processes are the main stimulators of the acute-phase proteins.¹³ Many types of cytokines can also play an important role in regulating other cytokines as well as the production of cytokine

receptors. For example, $\text{TNF-}\alpha$ is the main stimulator of interleukin-1 production in patients with rheumatoid arthritis; interleukin- 1β may increase or decrease expression of its own receptors; and interleukin-6 (IL-6) inhibits the expression of tumor necrosis factor alpha.¹³

In response to cytokines especially interleukin 6, acute-phase reactants or proteins are produced by the hepatocytes. Examples of acute phase proteins include fibrinogen, serum amyloid A (ORM), and CRP which have been examined as inflammatory markers related to the development of atherosclerosis.^{6,13}

Another form of protein is adhesion molecules, which is located on the cell surface and they are involved in adhesion with other cells or with the extracellular matrix. Every cell expresses cellular adhesion molecules. Adhesion molecules on the vascular endothelium and on circulating leukocytes mediate the recruitment of leukocytes in response to inflammatory stimuli. Increased expression of adhesion molecules along with immune cell migration inside the vessels initiates the development of atherosclerosis (figure 1.2).¹⁰

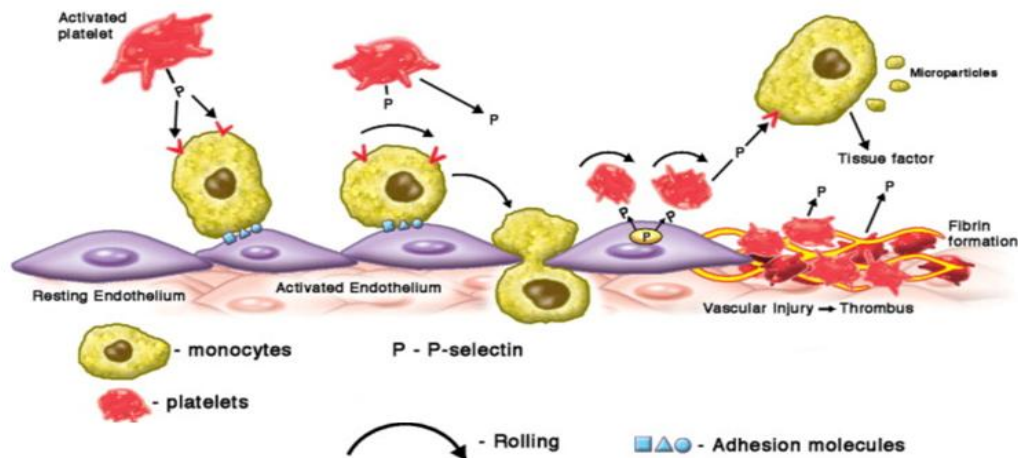


Figure 2. Inflammation and Atherosclerosis Development¹⁴

2.2 Menopause

Menopause is a natural process in women that is characterized by a progressive loss of ovarian activity and the cessation of menstrual periods, which is confirmed after 12 consecutive months without menses.¹⁵ In the American population, the mean age of menopause occurrence is 51.3 years.¹⁵ Menopause is associated with changes in plasma hormone levels, mainly a decrease in plasma estrogen, which is associated with changes in body composition (e.g. increased fat mass and decreased lean body mass) and body fat distribution (e.g. increased abdominal fat, especially visceral fat).^{2,11,15} Also, it has been recently reported that the menopause transition is associated with a decrease in physical activity energy expenditure and a shift towards a more sedentary lifestyle.⁷ This decrease in estrogen combined with the decrease in physical activity energy expenditure can increase the risk of weight gain, fat mass gain and abdominal fat accumulation among perimenopausal women.⁷ Similarly, it can also increase the risk of reductions in lean body mass, especially muscle mass (sarcopenia) as well as bone mineral density (osteoporosis).¹⁶ However, significant weight gain during menopause transition is not always observed. Previously by our group, we found that a decrease in physical activity energy expenditure was associated with a decrease in caloric intake in healthy none-obese premenopausal women that were followed for 5 years throughout their menopause transition.⁷

Experts at the Stages of Reproductive Aging Workshop working group in 2010 identified three stages that are important in a women's life (Table 1): 1) Reproductive stage, 2) Menopausal transition stage and, 3) Postmenopausal stage. The reproductive stage is characterized by the period from menarche (first menstrual cycle, or first menstrual bleeding) up to perimenopause. The menopausal transition is the interval after perimenopause and before postmenopause during which the ovaries progressively decrease the production and secretion of estrogen. The transition

interval is associated with irregular menstrual periods which are characterized by two skipped menstrual cycles with 60 or more days of amenorrhea. Finally, the postmenopausal stage corresponds to the occurrence of the final menstrual period and is then confirmed by 12 months of amenorrhea.¹⁵

Table 1. Menopause Staging System based on the Stage of Reproductive Aging Workforce.

Final menstrual period ↓							
Stages	-5	-4	-3	-2	-1	+1	+2
Terminology	Reproductive			Menopausal transition		Postmenopause	
	Early	Peak	Late	Early	Late*	Early*	Late
				Perimenopause			
Duration of Stage	Variable			Variable		4 years	Until demise
Menstrual cycles	Regular to variable	Regular		Variable cycle lengths	+2 skipped cycles	None	
Endocrine	Normal FSH		↑FSH	↑FSH		↑FSH	

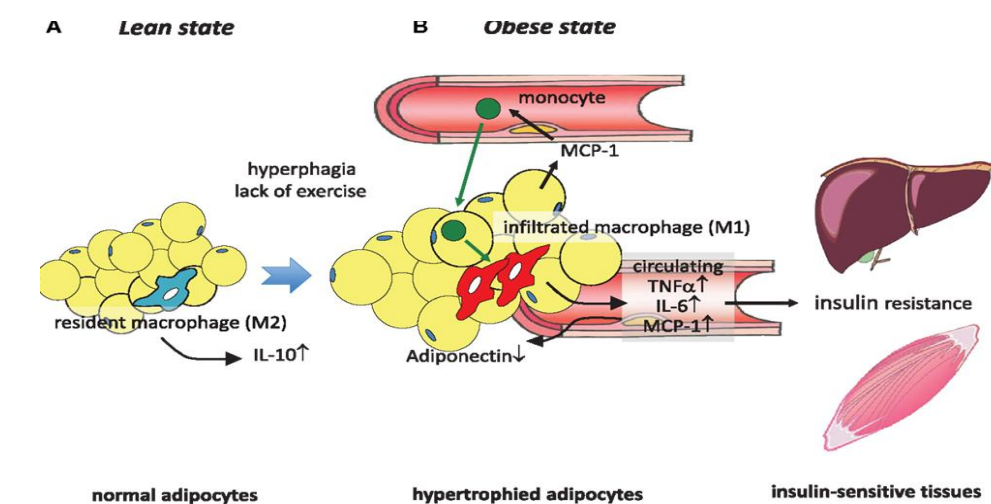
From Edwards 2013¹⁵

2.3 Menopause and inflammatory markers

There is evidence that menopause is associated with an increase in fat mass and abdominal adiposity even in the absence of significant weight gain.² The increase in fat mass across the menopausal transition may result in the development of a chronic sub-inflammation status.^{4,6}

Body fat distribution, especially visceral fat accumulation, is considered to be a key risk factor in the development of cardiometabolic disorders.^{4,6} In comparison to abdominal subcutaneous fat, visceral fat plays an important role in the development of the metabolic syndrome due to its proximity in respect to portal circulation.⁶ It has also been demonstrated that visceral fat adipocytes are more metabolically active than those in peripheral subcutaneous fat. As an endocrine organ, adipocytes secrete pro-inflammatory factors that contribute to the sub-chronic

inflammatory status.^{6,17} With increased fat mass and adipocyte hypertrophy (enlargement), the blood supply to adipocytes may be reduced. This leads to adipocyte hypoxia and necrosis.^{6,17} Adipocyte necrosis promotes macrophage infiltration, accumulation, and persistence in white adipose tissue. In response, resident macrophages in adipose tissue promote the development of a sub-chronic inflammation status. The primary cytokine responsible for the macrophage infiltration is the monocyte chemotactic protein-1 (MCP-1). Active macrophages express activation markers (MAC-2), a potent macrophage chemoattractant that promotes macrophage Aggregation, form crown like structures (CLS) that surround and scavenge residual adipocyte lipid, and ultimately form multinucleate giant cells (MGCs), a sign of chronic inflammatory state.¹⁷ Macrophages have two subclasses, M1 and M2 macrophages. M1 macrophages enhance a chronic inflammatory state whereas M2 macrophages participate in decreasing inflammatory responses from the adipose tissue. There is a shift from M2 macrophages to M1 macrophages with macrophage infiltration, which occurs as a result of less blood supply as well as adipocytes hypoxia and necrosis due to adipocyte hypertrophy. With the rise in M1 macrophages, cytokine production increases which leads to a more pro-inflammatory environment (figure 1.3).¹⁷



MCP-1: monocyte chemotactic protein-1; IL-6: Interleukin 6; IL-10: Interleukin 10; TNF- α : Tumor necrosis factor alpha

Figure 3. Fat Mass and Macrophage Infiltration¹⁸

Table 2. Present the summary of the evidences concerning the menopausal status and inflammatory markers.

Table 2. Menopausal Status and Inflammatory Markers

Author	Number of Subjects	Menopausal Status	Age (yrs)	Main Results
CROSS SECTIONAL STUDIES				
Puurunen (2011)¹⁹	50	Pre Post polycystic ovary syndrome	43 59	↑ CRP in Post
Yasui (2008)⁹	191 Peri (99) Post (92)	Healthy Peri and Post	47.9 ± 3.87 56.2 ± 7.13	↑ IL-4, ↑ MCP-1 in post. IL-1 β, IL-2, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, G-CSF, GM-CSF, TNF-α, INF-γ, MCP-1 ↔
Yasui (2007)²⁰	122 Pre (16) Peri (54) Post (52)	Healthy Pre, Peri and Post	40-65	↑ IL-2, ↑ IL-4, ↑ G-CSF, ↑ GM-CSF during menopause transition. IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p70), IL-13, IL-17, TNF-α, INF-γ, MIP-1β, (MCP)-1 ↔
Vural (2006)²¹	Pre (25) Post (24)	Healthy Pre and Post	43-55 19-38	↑ IL-4, ↑ IL-10, ↑ IL-12, ↑ TNF-α in post
Vural (2006)²²	75 Pre (25) Post (50)	Healthy Pre and Post (HRT, no HRT)	30 ± 2.5 48.6 ± 3.5	↑ TNF-α, ↑ IL-1β, ↑ IL-10 in post
Stefanska (2005)²³	Peri (47) Post (78)	Healthy Peri and Post	47 ± 3 53 ± 4	↑ sICAM-1, CRP, IL-6, fibrinogen, sVCAM-1 ↔
Karelis (2005)²⁴	88	Obese sedentary Post Metabolically Healthy obese, at risk	44-73	↓ CRP, ↓ alpha-1 antitrypsin in metabolic healthy obese
Sites (2002)²⁵	45 Pre 44 Post	Pre and Post	47 ± 3 51 ± 4	↑ TNF-α in post. CRP, IL-6 ↔
Cioffi (2002)²⁶	82 Pre 34 Post 48	Pre and Post	32 ± 7 54 ± 8	↑ IL-6, ↑ IL-18, ↓ TNF-α, ↓ TNF-β in post IL-2, IL-4, IL-5, IL-10, IL-12, IFN-γ ↔
Nishizawa (2002)²⁷	137 women 442 men	Pre and Post	53 ± 12	in post adiponectin ↔
Oger (2001)²⁸	Pre 74 Post 60	Healthy Prei and Post (HRT, no HRT)	45-54	↑ cICAM-1 in pos CRP e-selectin ↔
Kamada (2001)²⁹	97	Pre and Post (HRT, no HRT)	32-93	↑ TNF-α in post comparing to pre IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-18, IL-1α, IL-1β not detectable due to sensitivity of the assays used.

Giuliani (2001) ³⁰	220 Pre 59 Post 161	Pre and Post	25-104	↑ IL-6, ↑ sIL-6 in post
Hak (1999) ³¹	186 Pre 93 Post 93	Healthy Pre and Post	43-55	↔ CRP between pre & post
LONGITUDINAL STUDIES				
Lee (2009) ⁶	69	Healthy Peri follow up to post	45-44 follow up 49-69	↑ Adiponectin, ↑ SAA, ↑ MCP-1, tPA, IL-6, TNF-α, Leptin, CRP ↔
Wildman (2008) ³²	40	Healthy Pre, Peri, Post	39.6±0.6 46.3±0.6 51.8±0.6	↓ adiponectin during menopause transition, ↑ resistin in pre comparing to peri & post
Sowers (2008) ⁵	40	Obese and non obese Pre, Peri, and Post	38.5_52	↑ Resistin in pre comparing to peri & post in both groups. ↓ Adiponectin in peri comparing to pre & post status in both groups.
Matthews (2007) ³³	3249	Pre, early Peri, late Peri, Post (natural), Post (surgery), unknown status	46.3 ±2.7	↑ Fibrinogen in post and late peri comparing to pre, ↑ tPA-ag in early peri, late peri and post comparing to pre, CRP ↔
Sowers (2005) ³⁴	3286	Pre, early Peri, late Peri, Post (natural), Post (surgery), HT	42-52	Fibrinogen, tPA, CRP, PAI-1 in post and peri comparing to pre, ↔ ↑ CRP, ↓ PAI-1 in women with hormone therapy
Ridker (2000) ³⁵	366	Post with and without CVD	59.3	↑ CRP, ↑ IL-6, ↑ ORM, ↑ sICAM-1 in women with CVD CRP was related to intraabdominal fat in post

↓ Decrease; ↔ No change; ↑ Increase of Inflammatory Markers

Premenopausal (Pre), Perimenopausal (Peri), Postmenopausal (Post) women, Hormone Therapy (HT), Apolipoprotein B (APO-B), C-reactive protein (CRP), Interferon gamma (IFNγ), Interleukin (IL), Plasminogen activator inhibitor-1 (PAI-1), Serum Amyloid A (SAA) Soluble tumor necrosis factor receptor (sTNFR), Tumour necrosis factor (TNF-α), monocyte-chemotactic protein-1 (MCP-1), Tissue plasminogen activator (tPA), vascular cell adhesion molecule 1 (VCAM-1), Intercellular Adhesion Molecule 1 (ICAM-1), Granulocyte - colony stimulating factor (G-CSF), cardiovascular diseases (CVD)

In most cross-sectional studies during menopause, inflammatory markers do not differ or were higher in postmenopausal women compared to premenopausal women. However in few studies, inflammatory markers were lower in metabolically healthy obese postmenopausal women compared to premenopausal women.^{24,27} This discrepancy in the results of cross-sectional studies could be due to significant age gaps between cohorts (pre and postmenopausal women), for instance in a study by Giuliani *et al.*³⁰ there was up to 80 year gaps. In addition, other factors

such as body composition and the use of hormone therapy may have led to differences in inflammatory markers rather than the effects of women's menopausal status.⁶

The results of five longitudinal studies^{5,6,32-34} that investigated the influence of the menopause transition on inflammatory markers are not consistent. In the Study of Women's Health Across the Nation (Swan study) Matthews *et al.*³³ showed that menopausal status was an important predictor of all inflammatory markers, except for C-reactive protein. Compared with premenopausal women, late perimenopausal women had increased fibrinogen and plasminogen activator antigen (tPA-ag). tPA-ag and fibrinogen were also increased among postmenopausal women compared with premenopausal women.³³ Conversely, in the same study but with different grouping, Sowers *et al.*³⁴ showed that transitioning from premenopause and early perimenopause to postmenopause was not associated with significant change in levels of inflammatory factors. However, women who used hormone therapy showed 25% higher CRP concentrations and 20% lower PAI-1 concentrations compared with nonusers.³⁴ Difference in these results could be due to selection/grouping method used, such that i.e., in the Sowers *et al.* study, hormone therapy use and surgical menopause participant were taken into consideration.

In the Michigan Bone Health and Metabolism Study (MBHMS study)⁵, the researchers only measured resistin albeit they showed that levels of this inflammatory marker decreased in none-obese as well as in obese peri- and post-menopausal women compared to pre-menopause women. Resistin is secreted from adipocytes, but it is also produced by macrophages. It participates in regulation of systemic inflammatory response, stimulating the production of IL-6, IL-8, IL-12, and TNF- α in adipose tissue.¹ Researchers in MBHMS study suggested that it is possible resistin is produced in the ovary and declines with increasing ovarian age.⁵

Most longitudinal studies have not documented body composition changes such as body fat mass and abdominal fat which are important phenotype characteristics that could affect the production and the levels of inflammation markers in women going through the menopause transition.⁵ In one study that did evaluate body composition,³² the measurement technique that was used was bioelectrical impedance resistance, which has been questioned in terms of its measurement accuracy and reliability.³⁶ Considering the conflicting results and the lack of valid measurements of body composition changes in these longitudinal studies, the proposed study aims to provide more precise information on the relationship between body composition and inflammatory markers and their respective changes in women going through menopause transition as well as during the postmenopausal period.

2.4 Physical Activity and inflammatory markers

It has been shown that regular physical activity plays an anti-inflammatory role on adipose tissue and skeletal muscles.^{37,38} The following mechanisms have been suggested regarding how physical activity can reduce inflammation status:^{37,38}

- Increased anti-oxidant defense by up-regulation of anti-oxidant enzymes such as superoxide dismutase, glutathione peroxidase, glutathione reductase and catalases;
- Decreased reactive oxygen species and decreased nitric oxide activity, which are associated with a decrease in nuclear factor kappa B (an important transcription factor for inflammatory cytokines gene expression);
- Decreased adipocyte size and a reduction in hypoxia in adipose tissue;
- Improved endothelial function; and

- Increased cytokine release from exercising muscles, such as IL-6 which results in anti-inflammatory cytokine production of receptor antagonist (IL-10 and IL-1) and suppression of pro-inflammatory cytokines such as tumor necrosis factor alpha. The release of IL-8 from contracting muscles can play an important role in stimulating angiogenesis.³⁸

Table 3 provides a summary of the evidence regarding physical activity levels and inflammatory markers based on women's menopausal status.

Table 3. Physical Activity Levels and Inflammatory Markers base on Women Menopausal Status.

Author	Sample Size	Status	Age (yrs)	Study Design + measurement	Main Results
CROSS SECTIONAL STUDIES					
Lavoie (2010)³⁹	152	Obese overweight Post	56.7-58.3	Cross sectional study 3 groups PAEE high >1100 PAEE 730-1100 PAEE low <730 doubly labeled water combined with indirect calorimeter	↓ CRP, ↓ Haptoglobin in active women. Orosomucoid, IL-6, sTNFR1, TNF- α ↔
Autenrieth (2009)⁴⁰	796 (n = 359 women)	Overweight Men and women (66.3% post)	35-74	Cross sectional self report PA	↓ CRP, ↓ IL-6, ↓ fibrinogen in women
Woolf (2008)⁴¹	158	Healthy pre, peri, post	20-30 (n=49) 40-50 (n=62) >60 (n=47)	Cross sectional Self report + pedometer 7 days	lower level of Leptin in active vs. sedentary, CRP ↔
Mora (2006)⁴²	27158	Healthy women 54% post	mean 54.7	Cross sectional Questionnaire	Modest level of PA (at least 1000 kcal/wk or 2.5 hr/wk is significantly associated with more favorable level of CRP, Apo A1, fibrinogen, ICAM1-1
Manns (2003)⁴³	133	Post	50-73	Cross sectional Stanford 7-day activity recall	Higher PA, Lower CRP
LONGITUDINAL STUDIES					
Esposito (2003)⁴⁴	120	Pre obese healthy women	20-46	Randomized single-blind trial 24 mo occupational, household leisure time PA 1300d/first yr 1500d/2d yr	↓ IL-6, ↓ IL-18, ↓ CRP, adiponectin ↑

▼ Decrease; ↔ No change; ▲ Increase of Inflammatory Markers. Premenopausal (Pre), Perimenopausal (Peri), Postmenopausal (Post) women, Hormone Replacement Therapy (HRT), C-reactive protein (CRP), Interleukin (IL), Soluble tumor necrosis factor receptor (sTNFR), Tumour necrosis factor (TNF- α), Intercellular Adhesion Molecule 1(ICAM-1), physical activity (PA)
Results are based on women.

As demonstrated in Table 3, results of cross sectional studies have shown that physical activity levels are associated with lower inflammatory markers.³⁹⁻⁴³ Whereas three studies used self-report physical activity questionnaires, only two studies^{39,41} employed devices such as pedometers or methods such as indirect calorimeter and doubly labeled water for measuring daily physical activity. Finally, only one cross-sectional study compared the inflammatory marker profile of women based on menopausal status and it reported no significant difference in inflammatory markers between active and sedentary pre-, peri-, and postmenopausal women.⁴¹ To our knowledge there is only one longitudinal study to date on this topic and it compared levels of physical activity and inflammatory markers in premenopausal women over a two-year period. The results showed that higher physical activity levels and greater weight reduction via an intervention improved inflammatory profiles.⁴⁴ There is a lack of longitudinal research investigating the relationship between physical activity levels and inflammatory markers in women of different menopausal status.

2.5 Cardiorespiratory fitness and inflammatory markers

Studies have shown that cardiorespiratory fitness is inversely associated with inflammatory marker levels in men and women (Table 4). For example in a study by LaMonte,⁴⁵ CRP levels were lower in higher tertiles of cardiorespiratory fitness in 135 overweight women (age 55.0 $\pm$ 11.0 yr) with unknown menopause status. In another study by Giallauria⁴⁶ in 124 premenopausal women with polycystic ovary syndrome, cardiorespiratory fitness was inversely associated with CRP as well as fibrinogen. Similar results were reported by McGavock *et al.*⁴⁷ in

28 middle-aged women with type 2 diabetes for whom the menopause status was unknown. However, Valentine *et al.*⁴⁸ and Messier *et al.*⁴⁹ demonstrated that cardiorespiratory fitness was not significantly related to CRP levels in postmenopausal women. To our knowledge there are no longitudinal studies documenting the association between cardiorespiratory fitness levels and inflammatory markers in women of different menopausal status.

Higher levels of cardiorespiratory fitness, as achieved through regular exercise, have beneficial effects on cardiovascular health, thereby reducing the risk of coronary heart diseases, stroke, and mortality.⁵⁰ Exercise activates the hypothalamic–pituitary–adrenal axis.⁵¹ Through this axis, the sympathetic nervous system stimulates cortisol and adrenaline release from the adrenal cortex and medulla, respectively. These hormones inhibit pro inflammatory cytokines secretion such as TNF- α by monocytes. Exercise also promotes interleukin 6 secretion in the contracting skeletal muscles which can down-regulate TNF- α secretion leading to chronic decrease of pro-inflammatory markers. Exercise also increases plasma concentrations of key inflammatory immune cell chemokines (chemokine is a type of cytokine that is produced as a "chemo-attractant molecules" to attract cells to sites of inflammation); repeated increases of such chemokines may lead to a down regulation of their cellular receptors, and the result is reduced tissue infiltration⁵¹ which reduce chronic inflammation.

Table 4. Cardiorespiratory Fitness levels and Inflammatory Markers based on Menopausal Status.

Author	Number Subjects	Status	Age (yrs)	Study Design	Main Results
CROSS SECTIONAL studies					
Giallauria (2009)⁴⁶	124	Pre With polycystic ovary syndrome	24.1±4.5	Cross sectional	CRP, fibrinogen lower in women with higher CRF
Valentine (2009)⁴⁸	132 (47 men, 85 women)	Healthy post	70±5.4	Cross sectional	No relationship between CRP and CRF in women

Messier (2008) ⁴⁹	127	overweight and obese post	57.7±4.8	Cross sectional	No relationship between CRP and CRF in women
LaMonte (2002) ⁴⁵	135	Normal overweight	55±11	Cross sectional	CRP decreased across tertiles of Fitness

↓ Decrease; ↔ No change; ↑ Increase of inflammatory markers. Premenopausal (Pre), Perimenopausal (Peri), Postmenopausal (Post) women
month (mo), week (wk), aerobic training (AT), heart rate (HR), C-Reactive Protein (CRP), Cardiorespiratory Fitness (CRF)
Results are based on women participants. Note: No longitudinal studies were identified.

Menopause is characterized by the cessation of menstrual cycle due to loss of ovarian follicular activity and changes in body composition.⁵² These changes occur in the setting of progressive decreasing ovarian estrogen production, advancing age and changes in lifestyle that include decreased physical activity energy expenditure⁵³. Overall, in women, age-related changes in body composition, for example body fat distribution, body weight, body fat, waist circumferences and fat free mass changes, have been observed after menopause.⁵⁴ Aging also affect adipose tissue and muscle mass.⁵⁵ As menopause is a natural period in women's life, it is thus difficult to conceptually separate the independent effects of ageing from the effects of menopause. The general purpose of thesis is to determine the effects of menopause transition and duration of postmenopausal years on inflammatory markers. The influence of body composition, body fat distribution and physical activity energy expenditure will also be investigated. We hypothesized that women will have an increase in fat mass, waist circumference, inflammatory markers and a decrease in fat free mass, and physical activity energy expenditure. Also, number of years since final menstrual periods affects body composition, body fat distribution, physical activity energy expenditure and inflammatory makers.

3. Menopause, inflammatory markers and bone mineral density

Bone mineral density (BMD), which is expressed as grams of bone minerals per unit area, is the ratio of bone mineral content to bone size.⁵⁶

Previous studies have shown that a woman's hormonal profile is one of the major determinants of BMD.^{55,57,58} It is known that adequate estrogen levels stimulate bone formation, while estrogen deficiencies can result in bone loss.⁵⁷ Since menopause is associated with important changes in endocrine and metabolic processes, it has been suggested to have a strong impact on bone mineral density.^{58,59} Table 5 represents studies investigating bone mineral density and inflammatory markers based on menopausal status.

Bone remodelling is a continuous process that occurs throughout the life span and it is regulated by several factors such as genetics, levels of estrogen, calcium, and vitamin D. Body mass and body composition as well as physical activity are other important regulatory factors. In adults, the bone remodelling process occurs at a rate of about 10% per year. Bone homeostasis is controlled by a balance between bone resorption by osteoclasts and bone formation by osteoblasts cells. An imbalance between these cells could result in bone loss and osteoporosis.⁶⁰ Biological interactions between bone and the immune system occur via shared developmental pathways such as bone marrow. Indeed, bone marrow is the environment for the development of hematopoietic stem cells which are the blood cells from which all other immune system cells are derived.⁶¹ The differentiation of hematopoietic stem cells is regulated by immune cells. By producing pro-inflammatory cytokines, the immune cells have the potential to cause an imbalance in bone metabolism. These cytokines can increase bone resorption via activation of the receptors of nuclear factor kappa-B ligand (RANKL) which acts as a key factor for osteoclast

differentiation and activation. An example of this biological interaction between bone and immune system could be rheumatoid arthritis.⁶²

Menopause and the associated loss in estrogen can also affect bone metabolism as there is evidence that estrogen plays an important role on bone metabolism. Specifically, estrogen stimulates transforming growth factor beta (TGF- β) which inhibits osteoclast formation as well as osteoclast activity.⁶³ Moreover, the decreased concentration of oestrogen during the menopause transition favors bone resorption by stimulating pro-inflammatory cytokines such as IL-1, IL-6, and TNF- α . These inflammatory markers have a positive effect on pre-osteoclasts size in bone marrow which could lead to osteoporosis.⁶³

Given that previous studies have shown that levels of inflammatory markers are possibly higher in postmenopausal women compared to premenopausal women,³³ understanding the link between inflammation markers and bone metabolism in relation to the menopause status and estrogen deficiency would be important.

Table 5. Inflammatory Markers and Bone Mineral Density (BMD) and Content (BMC) base on Menopausal Status.

Author	Subjects (N)	Status	Age (yr)	Results
Koh (2005)⁶⁴	Pre=3662 Post= 1031	Healthy Pre and Post	30-76	↑ CRP in pre and post with osteopenia and/or osteoporosis comparing with normal BMD. CRP levels higher than 1.2 mg/l and 1.8 mg/l associated with osteoporosis and/or osteopenia in pre- and post
Papadopoulos (1997)⁶⁵	24	Healthy Post women	44-54	negative correlation between IL-6 and BMD. Women with high IL-6 levels= lower bone mass
Kania (1995)⁶⁶	45	Healthy Post women	25-74	No correlation between IL-6 and BMD at either the lumbar spine or femoral neck
Mckane (1994)⁶⁷	80	Normal Healthy Pre Post	24-87	No correlation between marker of inflammation (IL-6, IL1 alpha, IL-1 beta, IL-1ra)
Gertz (2010)⁶⁰	242 women	Healthy post	45.8-65	CRP, IL-1beta, IL-6, TNF-alpha accounted for 1.1–6.1% of the variance in the observed 12-mo changes in BMC and BMD

Ding (2008)⁶⁸	168	Post 48% women and men (normal overweight)	52-78	For both men and women= Change in total body BMD associated with baseline hs-CRP, IL-6, and TNF- α , and change in CRP ($r=-0.41$) and IL-6 ($r=-0.62$). associations between hs-CRP, change in hs-CRP and change in total body BMD in women more than or equal to 10 yr past menopause is more evident ($r=-0.58$, $P=0.001$; and $r=-0.48$, $P=0.01$, respectively). Same result for IL-6 ($r=-0.53$, $P=0.003$; and $r=-0.50$, $P=0.002$).
Scheidt-Nave (2001)⁶⁹	137	Post women	52-80	serum IL-6 is a predictor of post femur (not spine) bone loss. effect most relevant through the first postmenopausal decade
Abrahamsen (2000)⁷⁰	160	Peri women	50 $\pm$ 2.8	serum IL-1ra and sIL-6R associated with the rate of bone loss in peri. Serum IL-6 associated with an increase in lumbar spine BMD
Sponholtz (2014)⁷¹	2915 Men n=1293 Pre women n=231 Post women with HT n=498 without HT n=893	Post, pre women	29-86	Pre = inverse association IM and BMD (IL-6=-0.03, CRP= -0.015) but positive between TNF- α and BMD ($r=0.043$) Post = with HT positive association of CRP with BMD ($r=0.011$) without HT no association.

↑ Increase of Inflammatory markers, Premenopausal (Pre), Perimenopausal (Peri), Postmenopausal (Post) women . Bone mineral density (BMD), C-Reactive Protein (CRP), Interleukin (IL), Tumour necrosis factor (TNF- α)

Some cross sectional studies have revealed a significant relationship between BMD and inflammatory markers^{64,65} while some studies did not.^{66,67} Discrepancy in the results of cross-sectional studies could be due to significant age gaps between cohorts (pre and postmenopausal women). The relationship between bone loss and inflammatory makers has not been investigated in perimenopausal women. To our knowledge, five longitudinal studies have investigated the associations between changes in inflammatory markers and bone loss/resorption in women. Ding *et al.*⁶⁸ measured inflammatory markers at baseline and at 3-year follow-up in 168 postmenopausal women (48%) and men and found that baseline IL-6 and changes in IL-6 were negatively associated with BMD at the hip and spine respectively. Another study reported that serum IL-6 was a significant predictor of femoral (not lumbar spine) BMD loss in women, especially in the early postmenopausal period,⁶⁹ while Abrahamsen *et al.*⁷⁰ reported that serum IL-6 was associated with an increase in lumbar spine BMD over a 5-year period in

perimenopausal women. Overall, the literature on the association between inflammatory markers and bone loss in women is inclusive and requires further investigation.

CHAPTER 2

1. Specific Problem

The menopausal transition is a critical period that is associated with an increase in total body fat and a redistribution of body fat favoring an increase in abdominal fat, especially of visceral fat in normal weight, overweight and obese women.^{4,5} It is well documented that high levels of visceral fat is associated with an increased risk of developing metabolic syndrome, type 2 diabetes and cardiovascular diseases.^{4,5} In addition, increased fat mass and visceral fat in particular are associated with a sub-chronic inflammation state that is characterized by increased adipocyte size, hypoxia, macrophage infiltration and abnormal inflammatory markers secretion.⁴⁻⁶ However, there is a lack of evidence concerning the influence of menopause-related changes in body composition and body fat distribution as well as physical activity and cardiorespiratory fitness on inflammation markers. Also, little is known about the relationship between inflammatory markers with cardiometabolic risk factors and bone mineral density. Considering that the majority of the studies to date on this topic have been performed with overweight or obese none healthy women, further research is needed, especially longitudinal studies, that follows healthy, none-obese premenopausal women during the menopause transition as well as in the postmenopausal period.

Thus, our aim will be to determine the factors associated with the development of a chronic inflammation status and its relationships with cardiometabolic risk factors in non-obese healthy women. Also, we will investigate the relationship between inflammatory markers and bone mineral density in premenopausal women during the menopause transition and postmenopausal period.

2. Purpose

Primary Purpose:

Determine if body composition, body fat distribution, physical activity and cardiorespiratory fitness are significant predictors of inflammatory markers in non-obese healthy premenopausal women.

Secondary Purposes:

1. Determine the effect of menopause transition and physical activity energy expenditure on inflammatory markers.
2. Investigate the influence of inflammatory markers on bone mineral density in women going through the menopause transition.

Tertiary Purpose:

Determine the effects of duration of postmenopausal years on inflammatory markers. The influence of body composition, body fat distribution and physical activity energy expenditure will also be investigated.

3. Main Hypothesis

Primary hypothesis:

Waist circumference and physical activity energy expenditure are significant predictors of inflammatory markers in premenopausal women.

Secondary hypotheses:

1. Postmenopausal will show higher levels of inflammation markers than pre- and perimenopausal women. The inflammatory markers will be inversely associated with levels of physical activity energy expenditure.
2. Inflammatory markers will be inversely associated with level of bone mineral density.

Tertiary hypothesis:

We hypothesized women will have an increase in fat mass, waist circumference, inflammatory markers and a decrease in fat free mass, and physical activity energy expenditure. Also, number of years since final menstrual periods affects body composition, body fat distribution, physical activity energy expenditure and inflammatory makers.

CHAPTER 3

Method

Methods used in the present thesis are detailed within the methodology section of each article in chapters 4 to 6.

CHAPTER 4

EFFECT OF THE MENOPAUSAL TRANSITION AND PHYSICAL ACTIVITY ENERGY EXPENDITURE ON INFLAMMATORY MARKERS: A MONET GROUP STUDY

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No permission letter is needed from Wolters Kluwer Health, Lippincott Williams & Wilkins

(Menopause. 2016 Dec;23(12):1330-1338.)

Author contributions:

Éric Doucet, Rémi Rabasa-Lhoret, Jean-Marc Lavoie, Denis Prud'homme participated in the development of the research project (MONET). Jean-Philippe Bastard and Soraya Fellahi participated in the blood analysis and inflammatory makers measurements. Sahar Razmjou and Denis Prud'homme performed the analysis and interpretation and completed the writing of the manuscript. All authors were involved in the revision and interpretation of the paper.

Effect of the Menopause Transition and Physical Activity Energy Expenditure on Inflammatory Markers: A MONET Group Study

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Running title : Menopause and Inflammatory markers

Funding/support: This research was supported by the Canadian Institutes of Health Research (CIHR) (T 0602145.02), Denis Prud'homme is the recipient.

Financial disclosure/conflicts of interest: None reported.

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List of Abbreviations:

ApoB: Apolipoprotein B

Hs-CRP: C-reactive protein

HP: Haptoglobin

IL-8: Interleukin-8

IL-6: Interleukin-6

IL-1 β : Interleukin-1Beta

NF- κ B: Nuclear factor kappa B

ORM: Orosomucoid

sTNFR1 and sTNFR2: soluble TNF- α receptor 1 and 2

VO_{2peak} : Peak oxygen consumption

Abstract

Objective: Menopause transition is usually associated with changes in body composition and a decrease in physical activity energy expenditure. Adipose tissue, especially visceral fat, is an important source of inflammatory markers, which contributes to the development of a pro-inflammatory state. Conversely, high levels of physical activity and exercise have an anti-inflammatory effect. This study aimed to investigate the impact of menopause transition and physical activity on inflammatory makers. *Method:* One-hundred and two healthy premenopausal women participated in a 5-year longitudinal study. The present secondary analyses were performed on 58 participants with a full set of data (age: 49.6 ± 1.7 y; body mass index: 23.3 ± 2.4 kg/m²). Measures included body composition, waist circumference, fasting glucose and insulin levels, insulin sensitivity, plasma lipid levels, cardiorespiratory fitness, physical activity energy expenditure and inflammatory markers. *Results:* Repeated measure analyses revealed that, after the 5 year follow-up, significant increases in ferritin, interleukin-8 (IL-8), tumor necrosis factor- α soluble receptor 1 and 2 (sTNFR1 and sTNFR2) ($p < 0.001$), and a significant decrease in serum high-sensitive C-reactive protein (hs-CRP) ($p < 0.05$). Positive correlations were observed between change (year 5 – baseline) in waist circumference and changes in hs-CRP, orosomucoid (ORM), haptoglobin (HP) and apolipoprotein B (ApoB) levels ($0.26 \leq r \leq 0.34$; $p < 0.05$), and between change in peripheral fat and changes in ORM, ApoB, sTNFR2 ($0.28 \leq r \leq 0.39$; $p < 0.05$). On the other hand, negative correlations were found between change in physical activity energy expenditure and changes in ORM as well as ApoB ($r = -0.35$ and $r = -0.36$ respectively; $p < 0.05$). No significant correlations were found between change in cardiorespiratory fitness, glucose, insulin, insulin sensitivity and changes in inflammatory

markers. Multiple regression analysis showed that changes in physical activity energy expenditure and waist circumference together explained 23% of the individual variance of change in ORM ($p < 0.05$). Also, change in physical activity energy expenditure explained 15% ($p < 0.05$) of the variance of change in ApoB. Fat mass change explained 15 % ($p < 0.05$) of the variance of change in IL-8, and finally change in peripheral fat explained 15% of variance of change in sTNFR2 ($p < 0.05$).

Conclusion: The present study indicates that menopausal transition is accompanied by an increase in inflammatory markers, namely ferritin, IL-8, and sTNFR 1 and 2. The increase in IL-8 and sTNFR2 with menopause could be explained, in part, by changes in fat mass and peripheral fat, respectively.

Key words: Menopause transition, Physical activity, Inflammatory markers

Introduction

Menopause transition, a normal stage in a woman's life, is usually associated with changes in body composition, alterations in body fat distribution (e.g. increased abdominal fat, especially visceral fat)² and an increase in the prevalence of metabolic syndrome and cardiovascular diseases.^{11,15} Body composition changes during menopausal transition, including the increase in visceral fat, may contribute to the development of a pro-inflammatory adipokine state.⁶ Pro-inflammatory markers are important risk factors for the development of insulin resistance, metabolic syndrome, diabetes and cardiovascular diseases.^{35,72} Four of the most important and well-studied pro-inflammatory markers that are associated with an increased risk of developing chronic diseases are: high-sensitive C-reactive protein (hs-CRP), interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), and interleukin 1-beta (IL-1 β).

C reactive protein is an acute phase protein secreted by the liver in response to cytokine stimulation, such as IL-6. Both hs-CRP and IL-6 have been shown to be important markers of vascular inflammation and predictors of cardiovascular events.^{23,25} IL-6 also up-regulates TNF- α secretion.⁷³ TNF- α is a monocyte, macrophage-derived inflammatory marker that plays a critical role in insulin resistance in adipose tissue and muscle by impairing insulin signalling at the level of the insulin receptor substrate proteins.⁷⁴ TNF- α also activates the transcription factor nuclear factor kappa B (NF- κ B), which promotes pro-inflammatory cytokine secretion.⁷³ Finally, IL-1 β contributes to the development of cardiovascular diseases by promoting the expression of leukocyte adhesion molecules and enhancing adhesion of monocytes on the endothelial surface, thereby promoting the development of atherosclerosis.⁷⁵ IL-1 β also promotes the release of cytokines, such as IL-6, by activating macrophages.⁷⁵

Previous studies including the Montreal Ottawa New Emerging Team longitudinal study (MONET Study), from which the present sub-sample was drawn,² have reported that menopause transition is associated with an increase in total body fat and abdominal fat mass.^{76,77} This is associated with a decrease in physical activity energy expenditure and a shift to a more sedentary lifestyle.⁷ Furthermore, one study demonstrated that adipocyte enlargement contributed to the development of systemic inflammation by promoting pro-inflammatory cytokine secretion. Adipocyte hypertrophy is associated with a dramatic increase in macrophage infiltration into adipose tissue to scavenge dead adipocytes.⁷⁸ These findings suggest that adipocytes act as endocrine organ that produce inflammatory markers.⁷⁸ In contrast, cross-sectional studies have shown that higher reported levels of physical activity in adults were associated with lower plasma concentrations of inflammatory markers such as hs-CRP and IL-6.⁷⁹⁻⁸¹

There are inconsistencies in the literature with regard to the effects of menopause status or menopause transition on inflammatory makers. While several cross sectional studies have noted higher levels of inflammation in postmenopausal women,^{19,21,22,26,30} other studies reported no differences⁹ or lower levels²⁶ when compared to premenopausal women. Discrepancies among the findings of cross-sectional studies could be due to large mean age gaps (from 30 to 70 years) between cohorts (premenopausal and postmenopausal women) or to other factors such as differences in body composition or in the use of hormone therapy between study populations rather than the effects of menopause status *per se*.⁶

There are limited longitudinal data examining menopause-related changes in inflammatory markers. To our knowledge, there are currently only four available studies that have followed healthy women throughout menopause transition.^{5,6,33,34} Lees et al ⁶ showed deleterious changes in inflammatory markers in overweight women from the Seattle Midlife Women's Health Study

who were going through menopause transition. These changes in inflammatory markers were correlated with increased visceral adiposity. Using data from the Study of Women's Health Across the Nation, Matthews et al³³ showed that menopausal status was an important predictor of all inflammatory markers, except hs-CRP, in women across the weight-spectrum not using hormone therapy. In addition, compared with premenopausal women, late perimenopausal women had higher fibrinogen and tissue-type plasminogen activator antigen levels. Fibrinogen was also higher in postmenopausal women compared with premenopausal women.³³ In the Michigan Bone Health and Metabolism Study, the researchers measured only one inflammatory marker, resistin, and they showed that for normal weight and obese women, levels were significantly lower during perimenopause and postmenopause compared to the premenopause stage.⁵

Nonetheless, most of these longitudinal studies did not document, or account for changes in body composition. This is an important limitation since body fat and abdominal fat mass are important phenotypes that could affect inflammatory markers in women going through menopause transition.^{5,32} Moreover, these studies did not measure physical activity. Considering the conflicting results and the absence of detailed measurements of body composition and physical activity in women transitioning to menopause, the present study, which uses more rigorous phenotyping measures, may improve our knowledge by examining the effect of menopause transition on inflammatory markers, by accounting for body composition and physical activity. We hypothesized that menopause transition, would be associated with an increase in inflammatory markers. We also hypothesized that an increase in inflammatory markers would be positively associated with visceral fat and inversely associated with physical activity energy expenditure.

Methods

The present study is a secondary analysis of data from the MONET study. The complete design and methodology of the MONET study has been described previously.² Briefly, the MONET study was a 5-year longitudinal observational study investigating the effects of menopausal transition on body composition and cardiometabolic risk factors. One hundred and two premenopausal women (aged between 47 and 55 years) were recruited using community advertising and referrals from gynecology clinics. Analyses were conducted on 58 women who had complete data over the 5 year period for inflammatory markers and physical activity energy expenditure. Inclusion criteria for the study were the following: premenopausal status (two menstruations in the last 3 months, no increase in cycle irregularity in the 12 months before testing, and a plasma follicular-stimulating hormone level ≤ 30 IU/L (for verification); no surgically-induced menopause; non-smoker; body mass index between 20.0 and 29.9 kg/m²; and reported weight stability (± 2 kg) for 6 months before enrollment in the study. Exclusion criteria included: pregnancy or planning to become pregnant; medical problems that could have interfere with outcome variables including cardiovascular and/or metabolic diseases; use of oral contraceptives or hormonal therapy; high risk for hysterectomy; and history of drug and/or alcohol abuse. This study was approved by the University of Ottawa and the Montfort Hospital ethics committees. All participants signed a written consent form prior to their participation in the study.

Study Design- Participants visited the laboratory for multiple assessments each year over five years. The following measurements were made each year: anthropometry, body composition, body fat distribution, and fasting blood sampling for inflammatory markers, glucose and insulin

levels. Cardiorespiratory fitness was measured at year 1, 3 and 5. As a means of standardization, all laboratory measurements were performed in the early follicular phase (within 8 days) as long as women still had a regular cycle. Menopause status was determined yearly by self-reported questionnaire about menstrual bleeding and its regularity. Follicle stimulating hormone levels were measured annually during the early follicular phase to verify menopause status. Women were classified as premenopausal if they reported no change in menstrual cycle frequency and perimenopausal if they reported changes in menstrual frequency and/or amenorrhea for 3 to 11 months. Finally, women were classified as postmenopausal based on their final menstrual period and confirmed by 12 months of amenorrhea.²

Anthropometrics- Body weight and height were measured using a BWB- 800AS digital scale and a Tanita HR-100 stadiometer, respectively (Tanita Corporation of America Inc., Arlington Heights, IL). Body mass index was calculated as body weight in kilograms divided by height in square meters (kg/m^2). Waist circumference (mean of two measurements) was determined using a Gulick tape at the middle distance between the lowest rib and the iliac crest.^{2,82} Fat mass, % body fat, and fat-free mass were measured using dual-energy x-ray absorptiometry (GE-LUNAR Prodigy module; GE Medical Systems, Madison, WI) as previously described.^{83,84} Using duplicate measurements of % body fat in 12 healthy participants tested in our laboratory, the coefficient of variation and the correlation for the reproducibility were 1.8% and $r = 0.99$, respectively.

Cardiorespiratory fitness- An exercise stress test was performed to measure cardiorespiratory fitness. The participants were asked to refrain from vigorous exercise for 24 hours before the test and to refrain from consuming alcoholic beverages 6 hours prior. They were also asked to abstain from eating and drinking caffeinated beverages for 2 hours before the test. After a brief

warm-up, participants performed the progressive test protocol on a treadmill, which consisted of 3-minute stages with an increasing workload to the point of exhaustion. Heart rate, blood pressure, and the Borg scale⁸⁵ to evaluate participants' perceived exertion on a graded scale (6 to 20) were taken throughout the test at the end of each stage. The test was terminated when at least two of the following criteria were achieved: (1) predicted maximal heart rate was reached, (2) respiratory exchange ratio was greater than 1.1, (3) oxygen consumption remained stable or decreased with an increase in workload, or (4) rating on the Borg scale reached 19 or higher.⁸⁶ Peak oxygen consumption (VO_{2peak}) was considered to be the highest VO_2 reached during the test. Breath-by-breath samples of expired air were collected throughout the test, and measurements of VO_2 , carbon dioxide consumption, and respiratory exchange ratio were taken automatically using a Vmax 229 series metabolic cart (SensorMedics Corporation, Yorba Linda, CA). The indirect calorimetry unit was calibrated according to the manufacturer's specifications before every test.

Physical activity energy expenditure- An Actical accelerometer (Mini Mitter Co., Inc., Bend, OR) was used to measure daily physical activity and estimate mean physical activity energy expenditure. During 7 days following the annual lab visit, participants wore the accelerometer upon waking up and took it off just before going to bed. This time period was chosen because it is estimated to result in 90% reliability for the measurement of physical activity in both men and women.⁸⁷ The accelerometer was worn on the right hip (anterior to the iliac crest) and secured with an elastic belt with the arrow pointing up. The accelerometers used in this study were validated previously with the use of doubly labeled water measurements to estimate physical activity energy expenditure.⁸⁸

Blood sampling- Blood samples were taken after a 12-hour overnight fast. Plasma glucose levels were determined using spectrophotometric analysis after conversion of glucose to glucose-6-phosphate by hexokinase (Sigma-Aldrich Canada Ltd., Oakville, Ontario, Canada; Fisher Scientific Limited, Nepean, Ontario, Canada). Plasma insulin concentrations were determined by radioimmunoassay using ¹²⁵I-labeled human insulin and a human insulin antiserum (Millipore, St. Charles, MO). Insulin resistance was estimated using the QUICKI model (score) by the following equation: $1/[\log \text{ insulin (mU/l)} + \log \text{ glucose (mg/dl)}]$.⁸⁹ For hs-CRP, orosomucoid (ORM) and haptoglobin (Hp), assessments were made by immunonephelometry using an image analyzer (Beckman-Coulter, Villepinte, France) with detection limits of 0.20 mg/L, 0.25 g/L and 0.08 g/L respectively. Apolipoprotein B (apoB) was assessed by immunoturbidimetry (Architect, Abbott, Rungis, France) with detection limits of 0.03 g/L. Serum ferritin concentration was measured by chemiluminescence (Architect, Abbott, Rungis, France) with detection limits of 1 ng/mL. Serum IL-1 β , high sensitivity IL-6, IL-8, soluble tumor necrosis factor- α receptor 1 and 2 (sTNFR1 and sTNFR-2) were measured using standard and high sensitivity commercial enzyme-linked immunosorbent assay (ELISA) kits (Quantikine®, Abingdon, UK) with detection limits of 1.00 pg/mL, 0.04 pg/mL, 3.50 pg/mL, 0.77 pg/mL and 0.60 pg/mL, respectively. Coefficients of variation for inter- and intra-assay reproducibility were < 10% for inflammatory marker measurements performed with ELISA (IL-1 β , IL-6, IL-8, sTNFR1 and sTNFR2) and < 5% for other inflammatory markers, namely Ferritin, ORM, Hp, apoB and hs-CRP.

Statistical analysis- SPSS 16.0 for windows (SPSS Inc., Chicago, IL) was used to perform the statistical analyses. Variables were first checked for normality; hs-CRP, ferritin, IL-6 were log-transformed. IL-8 and IL-1 β were not normally distributed even after log transformation. Descriptive results are expressed as the mean $\pm$ standard deviation. A repeated-measure analysis

of variance was performed to determine absolute changes in variables of interest from baseline to year 5 and across menopausal status (perimenopause and postmenopause). However, one of the limitations in conducting analyses using year of study was the smaller representation, at certain time points, of data by menopause status. Therefore, additional exploratory analyses were conducted by restructuring the dataset to derive ‘cases’ or groups based on menopause status. Specifically, values in the first year were selected for premenopause; values for the last year in which the women were in perimenopause status were selected for perimenopause; and values of the fifth year were selected for postmenopause. An ANCOVA based on menopausal status controlling for time was conducted.²

In addition, given normally and non-normally distributed data, Pearson and Spearman correlations were performed, to examine the association between changes (year 5 – baseline) in body composition and body fat distribution indices, cardiorespiratory fitness, physical activity energy expenditure, glucose, insulin, QUICKI score and changes in inflammatory markers. Lastly, a stepwise multiple regression analysis was performed to identify the predictors of individual variation of changes in inflammatory makers. Variables included in the model were based on the correlations between the variables of interest and evidence in the literature. A p-value less than 0.05 was considered statistically significant.

Results

Baseline characteristics of the 58 participants included in the current secondary analysis are presented in Table 1 and are similar to the original MONET Study cohort of 102 healthy premenopausal women (data not shown). At baseline, all women were premenopausal. Their body mass index was categorized as below 29.9 kg/m² (average body mass index of 23.31±2.41).

They had a mean age of 49.66 ± 1.77 years. The average daily physical activity energy expenditure at baseline varied between 326.29 and 1904.71 kcal/day. By year 5, 2 (3.5%) women were still premenopausal, 15 (25.8%) were perimenopausal and 41 (70.7%) had become postmenopausal. To increase power we combined premenopausal and perimenopausal women for repeated measure analysis.

As presented in Table 2, results of the repeated-measure analysis of variance showed a significant effect of time for hs-CRP, ferritin, IL-8, sTNFR1, and sTNFR2 ($p < 0.05$), revealing increases overall, except for hs-CRP, which showed a decrease. There was also a significant effect of menopausal status on Hp levels, with perimenopausal women showing higher Hp levels compared to postmenopausal women ($p < 0.05$). Also, a significant effect of time by menopausal status interaction was observed for hs-CRP and Hp levels. After adjusting for waist circumference, the effect of menopause status on Hp levels remained significant. Furthermore, a significant effect of time by menopausal status interaction was noted for hs-CRP and Hp. However, the effect of time on inflammatory markers was no longer significant.

We also conducted additional analyses adjusting for age. Results showed a significant effect of time for ferritin, IL-8, sTNFR1 and sTNFR2. However, the effect of time and interaction on hs-CRP was no longer significant (results not shown).

The exploratory analysis of covariance revealed that perimenopausal and postmenopausal women had significantly higher levels of ferritin, IL-8, sTNFR1 and sTNFR2 than premenopausal women (Fig 1).

Pearson and Spearman correlations performed between the absolute changes (year 5 - baseline) in the independent variables and changes in inflammatory markers showed positive correlations between changes in waist circumference and changes in hs-CRP, ORM, Hp and ApoB levels

($0.26 \leq r \leq 0.34$; $p < 0.05$). Furthermore, a positive correlation was observed between changes in peripheral fat and changes in ORM, ApoB, and sTNFR2 ($0.28 \leq r \leq 0.39$; $p < 0.05$), whereas changes in daily physical activity energy expenditure were negatively associated with changes in ORM and ApoB ($r = -0.35$ and $r = -0.36$ respectively; $p < 0.05$) (Fig 2). Change in fat mass was positively correlated with changes in ApoB and sTNFR2 ($r=0.27$, $r=0.32$ respectively; $p < 0.05$) whereas changes in % body fat were negatively correlated with changes in IL-8 ($r = -0.29$, $p < 0.05$). No significant correlations were found between changes in glucose, insulin, QUICKI score and changes in the inflammatory markers (data not shown).

Multiple regression analyses (Table 3) were performed to model the predictors of absolute changes in inflammatory makers in premenopausal women transitioning to postmenopause based on the identified bivariate correlations as well as evidence in the literature. In separate regression analyses for each of the inflammatory markers, the following variables were used: fat mass, fat free mass, waist circumference, trunk fat, peripheral fat, cardiorespiratory fitness and physical activity energy expenditure. As a result, changes in daily physical activity energy expenditure and waist circumference together explained 23% of the individual variance in changes in ORM ($p < 0.05$). Also, change in daily physical activity energy expenditure explained 15% ($p < 0.05$) of the variance in change in ApoB while change in fat mass explained 15 % ($p < 0.05$) of the variance of IL-8. Change in peripheral fat explained 15% of the variance in change in sTNFR2 ($p < 0.05$).

Discussion

To our knowledge, this is the first longitudinal study that investigated changes in inflammatory markers among healthy, non-obese women going through the menopause transition. In fact, most studies to-date have been cross sectional^{9,19,21,22,26,30} and previous longitudinal studies involved mainly overweight or obese women with metabolic complications.^{6,33,34}

The main finding of the present study is that healthy normal weight women going through the menopause transition showed an increase in Ferritin, IL-8, sTNFR1 and sTNFR2. Our findings are contrary to those of Sowers et al³⁴ who observed no differences in inflammatory markers from premenopause to postmenopause in overweight women with and without hormone therapy. However, as previously reported,^{6,33,34} postmenopausal women tend to have either similar or higher circulating levels of inflammatory markers compared to premenopausal women. In the longitudinal Study of Women's Health Across the Nation, Matthews et al³³ showed that fibrinogen, and tissue-type plasminogen activator antigen were significantly higher in normal, overweight and obese perimenopausal and postmenopausal women in comparison to premenopausal women. Our findings are consistent with those from a cross-sectional study by Berge et al⁹⁰ who showed that ferritin levels were higher in healthy normal weight postmenopausal than premenopausal women. Such increases in ferritin are probably due to the cessation of regular vaginal bleeding that occurs progressively over the course of menopause. Previous studies have showed that ferritin levels could increase up to two to threefold after menopause transition.⁹¹

One possible explanation for the increase in inflammatory markers across the menopause transition could be related to the changing levels of estrogens during this period. Estrogens have the ability to suppress NF- κ B through a interaction of estrogens with a subunit of activated NF-

κ B.⁹² NF- κ B is a primary transcription factor and a key regulator for transcription of a number of inflammatory markers. Therefore, the loss of estrogen production with menopause could be associated with NF- κ B activation and upregulate the production of inflammatory markers⁹²

Surprisingly, we observed a reduction in hs-CRP levels over the course of this 5-year follow-up study. Previous research showed that hs-CRP is strongly related to waist circumference in healthy middle aged women³¹ and the fact that the increase in waist circumference over time in our study remained far below the waist circumference thresholds associated with poorer cardiometabolic health (mean waist circumference for our group was 77.9 ± 7.5 cm vs. reported thresholds of 88 cm)² may help to explain our lower hs-CRP. Furthermore, even if the participants in our MONET group study showed a decrease in their physical activity energy expenditure,⁷ they were still on average, more active than the general population.⁹³ Previous research indicates that higher daily physical activity energy expenditure is associated with reduced systemic inflammation (e.g. hs-CRP) in healthy women.³⁹

An additional explanatory factor might be the fibre intake of our participants. Studies have shown that high dietary fiber intake could decrease inflammation especially hs-CRP^{94,95} and women in our study had a mean fiber intake of 23.34 ± 7.79 g/d (measured by 7 day food dietary journal⁷) which is above dietary fiber recommendation by Health Canada (21 g/d)⁹⁶.

The fact that participants in the present study were healthy, normoglycemic, physically active women with a waist circumference below the risk threshold could have also contributed to our lack of association between the inflammatory markers and glucose, insulin and QUICKI score.

We observed positive correlations between body composition indices and inflammatory markers. The strongest positive associations were observed between changes in waist circumference and peripheral fat and changes in ORM and ApoB. Body composition and body fat distribution have

been shown to change during the menopause transition. In fact, the transition from premenopause to postmenopause is associated with increased fat mass and central fat, especially the accumulation of visceral abdominal fat.^{2,31} Results recently published by our group using the full cohort of women,² showed that although the participants did not show any significant change in body weight, they presented a significant increase in fat mass, % body fat and visceral fat². The same results were obtained in the present subsample.

With increased fat mass and adipocyte hypertrophy, the blood supply to adipocytes may be reduced, leading to adipocyte hypoxia and necrosis. As a result, macrophage infiltration occurs in adipose tissue.^{4,97} Macrophages are responsible for the expression of most of the tissue TNF- α and IL-6.^{5,97} TNF- α exerts its main effects via two high-affinity cell surface receptors. The extracellular domains of these receptors are sTNFR1 and sTNFR2. As the level of TNF- α increases, so too does the level of soluble receptors.⁹⁸ Measuring circulating levels of soluble receptors has been shown to be more reliable for determining TNF- α production and activity. In fact, our results showed that sTNFR 1 and 2 increase as women go through menopause transition. TNF- α is known to activate two transcription signaling pathways linked to insulin resistance; NF- κ B and the c-Jun NH2-terminal kinase pathways. TNF- α may also increase systemic insulin resistance by stimulating the release of fatty acids from adipose tissue into the circulation.⁸ Therefore, the increase in body fat mass observed across the menopausal transition in the present cohort, may favor the development of a pro-inflammatory adipokine profile such as an increase in TNF- α , IL-8 and ferritin.⁶ Ferritin, an acute phase reactant that stores iron, has been shown to increase up to threefold after menopause transition.^{42,90,91} Iron can promote oxidative stress, which is a deleterious factor leading to insulin resistance. In a subsample of the longitudinal Study of Women's Health Across the Nation, Kim and co-authors⁹⁹ showed that

ferritin, iron and insulin resistance increased as women went through menopausal transition. In their study, there was also a positive association between changes in iron levels over the course of the menopausal transition, and the changes in homeostatic model assessment-IR.⁹⁹ Although we also report an increase in ferritin levels over the menopause transition, we did not find an increase in insulin resistance or an association between ferritin and QUICKI score (data not shown).⁹⁹ The rise in ferritin is not surprising given its association with a reduction in vaginal bleeding during menopause transition.

Behaviorally, menopause is often associated with a shift to a more sedentary lifestyle and it has been shown that lower physical activity levels are related to higher levels of inflammatory markers.⁷ However, very few studies to date^{39,41} have employed devices such as pedometers or methods such as indirect calorimetry or doubly labeled water for measuring daily physical activity. Indeed, the majority of studies used self-report physical activity questionnaire rather than objective measure of physical activity such as accelerometers as used in the present study. Our results indicated that an increase in daily physical activity energy expenditure is associated with a decrease in ORM, ApoB and sTNFR2 (Fig 2). Our findings are in line with the results of previous cross-sectional studies^{39-41,43,99} which showed that high physical activity energy expenditure is associated with low levels of inflammatory markers. In addition, a prospective study by Esposito et al⁴⁴ reported that a low-energy Mediterranean-style diet and increased physical activity significantly decreased inflammatory markers in healthy obese women. This is no surprising given the data indicating that regular physical activity plays an anti-inflammatory role on the adipose tissue and skeletal muscles.^{41,44} The influence of daily physical activity energy expenditure on inflammation could be explained by: 1) increased anti-oxidant defense by up-regulation of anti-oxidant enzymes such as superoxide dismutase, glutathione peroxidase,

glutathione reductase and catalases; 2) decreased reactive oxygen species and decreased nitric oxide activity, which are associated with a decrease in NF- κ B (an important transcription factor for inflammatory cytokines gene expression); and 3) increased cytokine release from exercising muscles, such as IL-6 which results in anti-inflammatory cytokine production of receptor antagonist (IL-10 and IL-1) and suppression of pro-inflammatory cytokines such as TNF- α .^{37,38}

The findings of our study should be interpreted within certain limitations. First, the population studied was composed of healthy women with a body mass index of $23.31 \pm 2.41 \text{ kg/m}^2$. Therefore, our results are limited to this subgroup of the population. Still, it is noteworthy to point out that 45% of the women aged between 40 and 59 years in the Canadian population present a body mass index between 20 and 29 kg/m^2 . Second, the short and variable duration of the follow-up, especially for the postmenopausal period (only 2 years in most women) was another limitation, although 71% of the women became postmenopausal by year 5. Third, the number of women in the premenopausal group decreased throughout the study, which reduces power in conducting cross-menopause group comparisons. Finally, we used the more cost-efficient waist circumference and trunk fat by dual-energy x-ray absorptiometry instead of measuring visceral fat area by computed tomography.

Despite these limitations, the present study consists of a well-characterized cohort of women that were followed for 5 years. The longitudinal design allowed us to compare the same women as they went through the menopause transition, and thus limit intra-individual variation in factors that could account for the effects on inflammatory markers. Lastly, we used gold-standard methods for the measurement of body composition (dual-energy x-ray absorptiometry) and daily physical activity energy expenditure (accelerometer).

Conclusion

The present study indicates that menopausal transition is accompanied by an increase in some inflammatory markers, especially ferritin, IL-8, and sTNFR 1 and 2. The increase in IL-8 and sTNFR2 with menopause might be partly explained by changes in fat mass and peripheral fat, respectively.

Acknowledgments

Remi Rabasa-Lhoret is supported by a FRQS (Fonds de Recherche en Santé du Québec) senior scholarship. Eric Doucet is the recipient of CHIR/Merck-Frosst New Investigator Award, a Canadian Foundation for Innovation New Opportunities Award, and an Early Research Award (Ontario). We thank Beckman-Coulter (France) for the kind gift of antibodies to perform hs-CRP, orosomucoid and haptoglobin measurements. We thank Dr Kristi Adamo and Dr Eva Guérin for her assistance in editing the manuscript.

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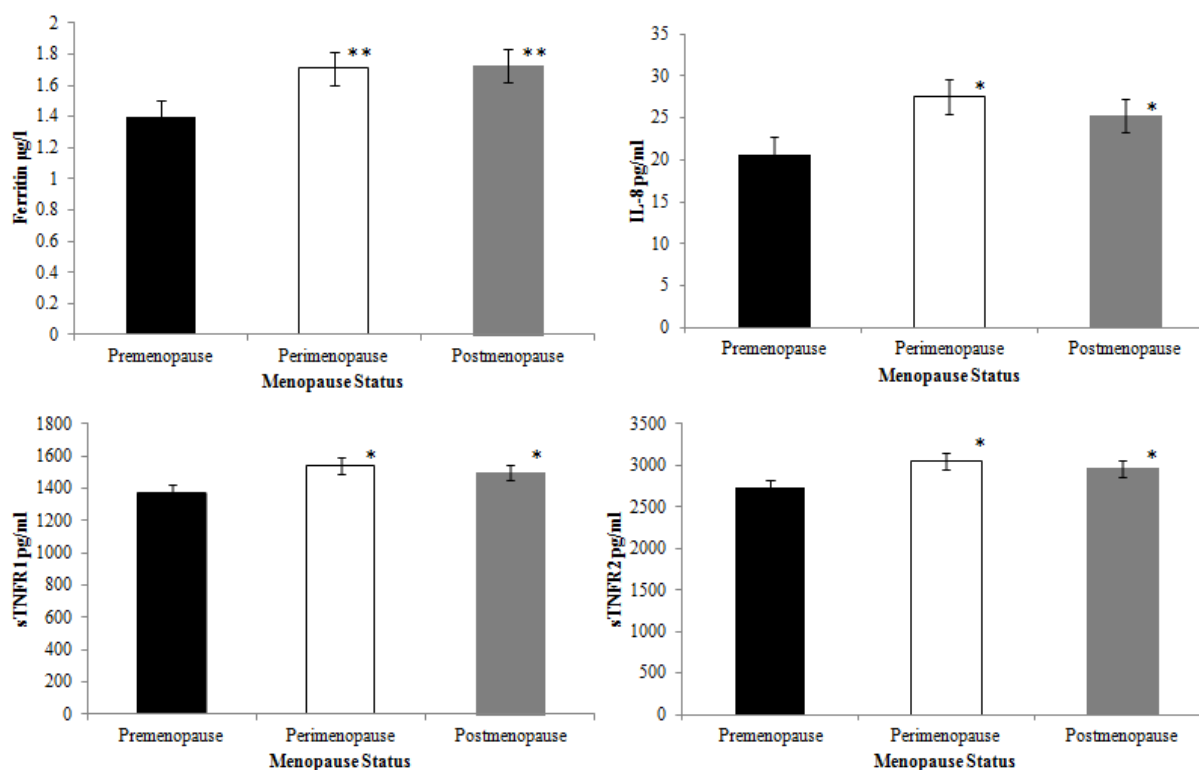


Figure 1. The effect of menopause status on inflammatory markers adjusted for time. *Significantly different from premenopause ($P < 0.05$); **Significantly different from premenopause ($P \leq 0.001$); IL-8, Interleukin 8; sTNFR1,2, Soluble tumor necrosis factor- α receptor 1 and 2.

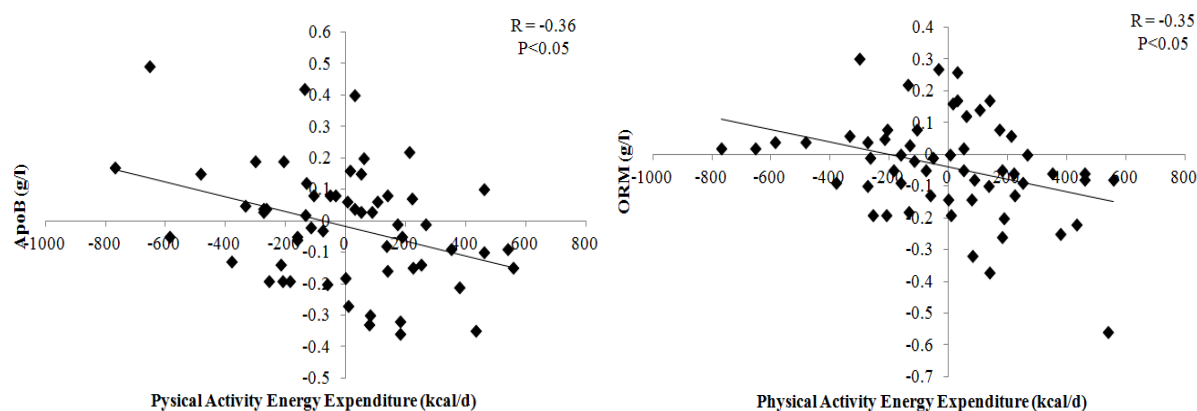


Figure 2. Pearson correlation between changes in daily physical activity energy expenditure and inflammatory markers. ORM, orosomucoid; ApoB, apolipoprotein B.

Table 1. Baseline characteristics of the premenopausal women (n=58)

Variable	Mean $\pm$ SD	Range
Age (years)	49.66 $\pm$ 1.77	47.00-54.00
Weight (kg)	60.58 $\pm$ 6.58	46.80-76.60
Height (cm)	161.31 $\pm$ 6.94	150.00-180.50
Body mass index (kg/m ²)	23.31 $\pm$ 2.41	19.27-28.75
Waist circumference (cm)	78.25 $\pm$ 7.15	62.2-93.7
% Body fat	31.60 $\pm$ 7.07	18.2-41.7
Fat mass (kg)	19.25 $\pm$ 5.59	9.63-29.95
Fat-free mass (kg)	38.46 $\pm$ 3.98	31.16-47.32
Peripheral fat (kg)	9.99 $\pm$ 2.76	4.98-15.54
Trunk fat (kg)	9.26 $\pm$ 3.24	3.33-18.27
VO_{2peak} (ml O ₂ /kg/min) ^a	33.70 $\pm$ 6.79	20.90 - 52.00
PAEE (kcal/day)	831 $\pm$ 282	326-1904
Glucose (mmol/L)	4.81 $\pm$ 0.39	3.80 - 5.70
Insulin (μ Um/L)	11.87 $\pm$ 4.04	5.03 - 32.07
QUICKI	0.33 $\pm$ 0.01	0.29 - 0.38

^a(n =56); PAEE, Physical activity energy expenditure.

TABLE 2. Inflammatory markers by time and menopause status.

	Perimenopause		Postmenopause		ANOVA-Repeated Measure		
	Baseline	Year 5	Baseline	Year 5	Time	Status	Interaction
n	17*	17*	41	41			
hsCRP, mg/l	2.51±2.42	1.16±1.03	1.27±1.43	1.26±1.87	<0.05	NS	<0.05
ORM, g/l	0.68±0.16	0.58±0.16	0.61± 0.10	0.60±0.17	NS	NS	NS
Hp, g/l	1.09±0.41	0.90±0.33	0.77±0.27	0.77±0.36	NS	<0.05	<0.05
ApoB, g/l	0.79±0.17	0.75±0.19	0.73±0.13	0.73±0.20	NS	NS	NS
Ferritin, µg/l	30.94±24.17	61.17±72.40	37.26±30.88	71.92±37.71	<0.05	NS	NS
IL-6, pg/ml	0.87±0.47	0.94±0.67	1.09±1.36	1.12±0.94	NS	NS	NS
IL-8, pg/ml	18.83±3.86	26.56±13.86	21.46±12.40	25.27±11.26	<0.05	NS	NS
IL-1B, pg/ml	4.42±2.96	4.61±4.57	3.40±1.03	3.43±1.20	NS	NS	NS
sTNFR1, pg/ml	1339.21±240.20	1534.19±219.50	1381.89±231.37	1497.93±260.00	<0.05	NS	NS
sTNFR2, pg/ml	2762.02±449.37	3063.10±750.10	2691.22±454.40	2968.18±629.21	<0.05	NS	NS

Values are mean ± SD. Hs-CRP, high sensitive c-reactive protein; ORM, orosomucoid; Hp, haptoglobin; ApoB, apolipoprotein B; IL-6, Interleukin 6; IL-8, Interleukin 8; IL-1B, interleukin 1B; sTNFR1,2, Soluble tumor necrosis factor –α receptor 1 and 2; NS, not significant. Log transformation scores for CRP, Ferritin, IL-6 have been used for analysis but physiological values have been reported. IL-8, IL-1B Wilcoxon method has been used. *two pre-menopausal woman were added to the perimenopause group.

Table 3. Determinants of changes in inflammatory markers using multiple regressions analysis.

Stepwise regression	Dependent variable	Independent variable	R ²	Total R ²	P value
	ORM (N=54)	Physical activity energy expenditure	0.15	23%	0.003
		Waist circumference	0.08		0.025
	ApoB (N=54)	Physical activity energy expenditure	0.15	15%	0.003
	IL-8 (N=55)	Fat mass	0.15	15%	0.004
	sTNFR2 (N=55)	Peripheral fat	0.15	15%	0.004

Variable used in the regression: Fat mass, Fat free mass, Waist circumference, Trunk Fat, Peripheral Fat, VO_{2peak} , Physical activity energy expenditure. ORM, orosomucoid; ApoB, apolipoprotein B; IL-8, Interleukin 8; sTNFR2, Soluble tumor necrosis factor-α receptor 2.

CHAPTER 5

ASSOCIATION BETWEEN INFLAMMATORY MARKERS AND BONE MINERAL DENSITY IN PREMENOPAUSAL WOMEN GOING THROUGH MENOPAUSE TRANSITION: A MONET STUDY

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(Manuscript submitted to Archives of Osteoporosis Journal Submission ID: OSIN-D-17-00838)

Author contributions:

Éric Doucet, Rémi Rabasa-Lhoret, Isabelle Dionne, Denis Prud'homme participated in the development of the research project (MONET). Jean-Philippe Bastard and Soraya Fellahi participated in the blood analysis and inflammatory makers measurements. Sahar Razmjou and Denis Prud'homme performed the analysis and interpretation and completed the writing of the manuscript. All authors were involved in the revision and interpretation of the paper.

Association Between Inflammatory Makers and Bone Mineral

Density in Premenopausal Women Transitioning to Menopause: A

MONET STUDY

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Funding/support: This research was supported by the Canadian Institutes of Health Research (CIHR) (T 0602145.02) grant received by Dr. Denis Prud'homme.

Financial disclosure/conflicts of interest: None reported.

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Abstract

INTRODUCTION: Progressive estrogen loss during the transition to menopause is associated with higher levels of inflammatory markers that could promote bone loss.

PURPOSE: To investigate the association between inflammatory markers and bone mineral density (BMD) in premenopausal women transitioning to menopause.

METHOD: The study included 102 healthy premenopausal women at baseline (age: 49.93 ± 1.90 y; BMI: 23.27 ± 2.24 kg/m²) who took part in a 5-year longitudinal study on the effects of the menopause transition on body composition and cardiometabolic risk factors. Outcome measures included body composition (fat mass and fat free mass) and BMD (total, lumbar spine and femoral neck) measured by dual-energy x-ray absorptiometry as well as waist circumference, cardiorespiratory fitness, physical activity energy expenditure, and a panel of inflammatory markers.

RESULT: A significant decrease in BMD (total, lumbar spine and femoral neck) was observed from year 1 to 5 (all $p < 0.001$). Longitudinal data analysis showed no significant association between change (% Δ) of inflammatory markers and % Δ of BMD. However, a positive correlation was found between baseline hs-CRP levels ($r=0.37$) and haptoglobin (Hp) ($r=0.24$) and % Δ of total BMD whereas a negative correlation was observed with interleukin-6 (IL-6) ($r=-0.26$) (all $p < 0.05$). In addition, a positive correlation was found between baseline hs-CRP ($r=0.32$) and Hp ($r=0.27$) (all $p < 0.05$) and % Δ of lumbar spine BMD. Finally, there was a positive correlation between baseline hs-CRP ($r=0.31$, $p < 0.05$) and % Δ of femoral neck BMD. Stepwise regression analysis showed that baseline levels of hs-CRP, IL-6 and menopause status explained 35% of individual variations of % Δ of total BMD in women transitioning to menopause. Baseline levels of Hp and menopause status explained 17% of individual variation

of % Δ at lumbar spine BMD. Also, 22% of variation in % Δ femoral neck BMD was explained by baseline hs-CRP and femoral neck BMD and % Δ of physical activity energy expenditure.

CONCLUSION: We did not observe an association between % Δ in inflammatory markers and % Δ in BMD in women transitioning to menopause. However, baseline levels of hs-CRP, Hp and IL-6 are among the predictors of individual variation of bone loss in women transitioning to menopause. Longitudinal studies with a larger sample size need to be performed to confirm our results on the role of systemic inflammation in bone loss in women transitioning to menopause.

Keywords: bone mineral density, cytokines, inflammation, menopause

Word Count in Abstract: 373

Number of Tables: 3

Number of figure: 1

Conflict of interest: The authors report no conflicts of interest.

This research was supported by the Canadian Institutes of Health Research (T 0602145.02).

Introduction

Osteoporosis is a metabolic bone disease characterized by compromised bone strength and increased risk of bone fractures.¹⁰⁰ As much as one and a half million (10%) Canadians over the age of 40 years old having osteoporosis. Women are four times more likely to have osteoporosis than men.¹⁰¹ The overall yearly cost of treating osteoporosis and consequent fractures is over \$2.3 billion in the Canadian health care system.¹⁰¹

Studies show that factors such as weight, body composition, physical activity level and systemic inflammation contribute to regulating bone mass homeostasis throughout a woman's life.^{63,102,103}

Bone mass homeostasis is the result of a balance between bone formation by osteoblasts and bone resorption by osteoclast cells. An imbalance between the activity of these cells toward excessive resorption results in bone loss and osteoporosis.¹⁰⁴ One of the major factors increasing the risk of osteoporosis in women is the low levels of estrogen associated with the natural process of aging observed in postmenopausal years. Estrogen stimulates the release of transforming growth factor beta (TGF- β), an inhibitor of bone resorption that decreases osteoclast formation as well as osteoclast activity.⁶³ The progressive decrease of estrogen levels during the transition to menopause could cause bone resorption by stimulating inflammatory markers production such as C reactive protein (CRP), interleukin 1-beta (IL-1 β), interleukin 6 (IL-6), interleukin 8 (IL-8), and tumor necrosis factor alpha (TNF- α).⁶³ As a result, these pro-inflammatory cytokines could promote activation of receptor activator of nuclear factor - κ B ligand (RANK), which increases osteoclast differentiation and activity, stimulating bone loss.^{63,103,105,106} Another inflammatory marker, ferritin, an iron storage element, plays a role in upregulating osteoclast activity by promoting mitochondrial biogenesis. Moreover, it suppresses

osteoblast differentiation and activity.^{107,108} Recently, there has been clinical evidence to support an association between levels of ferritin and bone mineral density (BMD).¹⁰⁹

Moreover, studies show that reduced physical activity levels during menopause⁵³ could affect bone loss.^{110,111} Physical activity promotes bone strength^{110,111} and studies suggest higher physical activity levels are associated with lower levels of inflammatory markers and as a result, lower rates of bone loss.^{39,112,113} Physical activity plays a role in down regulating nuclear factor- κ B (NF- κ B) (an important transcription factor for inflammatory cytokine gene expression).¹¹³

Scientific evidence on the link between inflammatory markers and BMD is based mostly on results of cross-sectional studies. Some studies suggest a negative relationship between levels of inflammatory markers and bone mineral density in pre-^{64,114} and postmenopausal women,^{65,115} while others reported no relationship.^{66,67,116} Longitudinal studies are more reliable than cross-sectional studies because they report changes of the same group of participants and make population-based inferences.¹¹⁷ Only two longitudinal studies^{68,70} have investigated the relationship between levels of inflammatory markers and change in BMD. Ding *et al.*⁶⁸ found among 193 women with an average 2.9 year follow-up (age range: 52-78 years old) that both baseline IL-6 serum levels, and change in IL-6 levels were consistently associated with bone loss (total, lumbar spine, and total hip) whereas the effects of TNF- α and CRP were not independent of IL-6. In contrast, in a five-year longitudinal study Abrahamsen *et al.*⁷⁰ reported in 160 perimenopausal women (age: 50.1 ± 2.8 years), that baseline IL-6 serum levels were not associated with bone loss whereas serum levels of IL-1ra and sIL-6R were associated with the rate of bone loss.

Considering that previous studies have showed that levels of inflammatory markers are higher in postmenopausal women as compared to premenopausal women⁴ and the inconclusive results of

longitudinal studies^{68,70} on the potential role of inflammatory makers on BMD loss during the transition to menopause, additional prospective studies are needed. Thus, the objective of this study was to determine the association between inflammatory markers and BMD in healthy normal weight premenopausal women transitioning to menopause. We hypothesized that inflammatory markers are negatively associated with BMD loss during the transition to menopause.

Methods

The present study is a secondary analysis of data from the 5-year longitudinal study performed by the Montreal-Ottawa New Emerging Team (MONET) (from 2004 to 2009) regarding the effects of menopausal transition on body composition and cardiometabolic risk factors. All experiments were conducted according to the guidelines laid down in the Declaration of Helsinki and all the procedures involving human participants were approved by the University of Ottawa and Montfort Hospital ethics committees. Written informed consent was obtained from all participants. The complete study design and methodology of the MONET study is described in a previous publication.²

Participants- One hundred and two premenopausal women (aged between 47 and 55 years) were recruited using community advertising and referrals from gynecology clinics. Inclusion criteria for the study were the following: premenopause status (two menstruations in the last 3 months, no increase in cycle irregularity in the 12 months before testing, and a plasma levels of follicular-stimulating hormone ≤ 30 IU/L as a mean of verification); no surgically induced menopause; non-smoker; body mass index (BMI) between 20.0 and 29.9 kg/m²; and reported weight stability (± 2 kg) for 6 months or more before enrolment in the study. Exclusion criteria included: pregnancy or planning to become pregnant; medical problems that could have interfered with outcome variables including cardiovascular and/or metabolic diseases; taking oral contraceptives or replacement hormonal therapy; high risk for hysterectomy; and history of drug and/or alcohol abuse.

Status- Yearly menopause status was determined by self-reported questionnaire about menstrual bleeding and its regularity. Plasma levels of follicle-stimulating hormone were measured during the early follicular phase to verify menopause status. Women were classified as premenopausal if

they reported no change in menstrual cycle frequency and perimenopausal if they reported changes in menstrual frequency and/or amenorrhea for 3 to 11 months. Finally, women were classified as postmenopausal based on their final menstrual period and confirmed by 12 months of amenorrhea¹¹⁸.

Study Design- Participants came annually to the laboratory for an evaluation of the following measures: anthropometric, body composition and body fat distribution, BMD, physical activity energy expenditure, cardiorespiratory fitness (measured at year 1, 3 and 5) and inflammatory markers (measured at baseline and at year five). As a means of standardization, all measurements were performed in the early follicular phase (within 8 days) as long as women still had a regular menses cycle.

Anthropometric assessment- Body weight and height were measured using a BWB- 800AS digital scale and a Tanita HR-100 height rod, respectively (Tanita Corporation of America Inc., Arlington Heights, IL). BMI was calculated (body weight in kilograms/height in square meters (kg/m^2)). Waist circumference (mean of two measures) was determined using a Gulick tape at the middle distance between the lowest rib and the iliac crest.⁸² Body composition (fat mass, % fat mass and fat-free mass) and BMD (lumbar spine [L4-L5] and femoral neck) were measured using dual-energy x-ray absorptiometry (DXA) (GE-LUNAR Prodigy module; GE Medical Systems, Madison, WI) as previously described.⁸³ The coefficient of variation and the correlation for % body fat measured in 12 healthy participants tested in our laboratory were 1.8% and the $r = 0.99$, respectively.² Meanwhile the coefficient of variation for measuring BMD by DXA was <1% using PHANTOM. The participants wore a light hospital gown without shoes during these measurements.

Cardiorespiratory fitness - A progressive exercise stress test was performed on a treadmill to measure the participant's cardiorespiratory fitness. Heart rate and blood pressure were measured at rest and at the end of each 3-minute stage, with the addition of the Borg scale during the test. The Borg scale is a category scale for ratings of perceived exertion ranging from 6 to 20.⁸⁵ Breath-by-breath samples of expired air were collected through a mouthpiece during the test and measurements of V_{O_2} and V_{CO_2} were obtained using a V_{max} 229 Series Metabolic Cart (SensorMedics Corporation, Yorba Linda, CA). The indirect calorimetry unit was calibrated according to the manufacturer's specifications before every test. The exercise stress test was terminated when at least two of the following criteria were achieved:⁸⁶ predicted maximal heart rate for age was reached; respiratory quotient was greater than 1.1; oxygen consumption remained stable or decreased with an increase in workload; or rate of Borg-type scale reached 19 or higher. Peak oxygen consumption ($V_{O_2 \text{ peak}}$) was considered as the highest oxygen consumption reached during the test.

Physical activity energy expenditure- An accelerometer (Actical; Mini Mitter Co., Inc., Bend, OR) was used to measure physical activity and to estimate physical activity energy expenditure. During 7 days, the participants wore the accelerometer on their right hip (anterior to the iliac crest), secured with an elastic belt with the arrow pointing up, upon waking up and took it off just before going to bed. This duration was chosen because it is estimated to result in 90% reliability for the measurement of daily physical activity in both men and women.⁸⁷ The accelerometers used in this study were validated previously with the use of doubly labeled water measurements to estimate daily physical activity energy expenditure.⁸⁸

Blood sampling- Fasting samples were taken after a 12-hour overnight fast. Plasma levels of high sensitive (hs)-CRP and Hp were assessed by immunonephelometry using an image analyzer

(Beckman-Coulter, Villepinte, France) with detection limits of 0.20 mg/L and 0.08 g/L respectively. Plasma levels of ferritin was measured by chemiluminescence (Architect, Abbott, Rungis, France) with detection limits of 1 ng/mL. Plasma levels of interleukin IL-1 β , IL-6, IL-8, soluble receptor tumor necrosis factor 1 (sTNFR1) and 2 (sTNFR2) were measured using standard and high sensitivity commercial ELISA kits, respectively (Quantikine®, Abingdon, UK) with detection limits of 1.00 pg/mL, 0.04 pg/mL, 3.50 pg/mL, 0.77 pg/mL and 0.60 pg/mL respectively. Sample below the limit of inflammatory marker detection (varying from 4 to 16% depending of the inflammatory marker) were assigned a zero value.^{119,120}

Statistical analysis

SPSS 16.0 for Windows (SPSS Inc., Chicago, IL) was used to perform the statistical analyses. Variables were first checked for normality of distribution, and hs-CRP, ferritin, IL-6, IL-8 and IL-1 β were log-transformed. However, IL-8 and IL-1 β were still not normally distributed thus we used non-parametric analysis for these variables. Results are expressed as mean $\pm$ standard deviation. A repeated-measure analysis of variance was performed to determine if relative change in BMD [(year 5 – year 1)/year 1 $\times$ 100] was significantly different depending on menopausal status. Partial correlations were performed to examine the association between inflammatory markers and bone mineral density in premenopausal women and their respective relative changes after the five-year follow-up. In addition, a stepwise regression analysis was performed to identify the predictors of the individual variation of BMD loss during the transition to menopause. P values less than 0.05 were considered statistically significant.

Results

From the 102 Caucasian women who were recruited, 91 completed the study. Eleven women with similar baseline characteristics dropped out of the study. They were either not interested or did not accept the DXA risk. The characteristics of participants are presented in Table 1. At baseline, participants were all premenopausal, non-obese based on BMI and had normal BMD values at lumbar spine and femoral neck. After the 5-year follow-up, 4% (n=4) of participants were still premenopausal, 29% (n=26) perimenopausal and 67% (n=61) postmenopausal. At baseline, none of the women were using hormone replacement therapy. However, during the 5-year longitudinal study, five women started using hormone replacement therapy. There were no significant differences in the cardiometabolic characteristics, body composition profile, bone density or inflammatory markers level between hormone therapy users and nonusers (results not shown).

We observed a significant decrease in total BMD as well at lumbar spine and femoral neck over the 5-year follow-up (Table 2). No significant effect of menopausal status was observed but time and menopausal status significantly affected total, lumbar spine and femoral neck BMD. As previously reported ¹²¹, levels of hs-CRP decreased ($p<0.05$) while IL-8, ferritin, sTNFR1 and sTNFR2 levels increased ($p<0.05$) and IL-6 and IL-1 β levels remained unchanged over the 5-year follow-up. Hp levels decreased significantly ($p<0.05$) (data not shown).

Partial correlations between baseline inflammatory markers and baseline and percent change BMD are present in Table 3. Baseline analysis showed only negative associations between the levels of hs-CRP and total, lumbar spine and femoral neck BMD at baseline. After controlling

for body weight, cardiorespiratory fitness and physical activity energy expenditure, correlations were still significant ($p < 0.05$).

Furthermore, we found significant correlations between baseline hs-CRP, Hp, and IL-6 levels and percent decrease of total BMD over the 5-year follow-up (table 3). Hs-CRP and Hp levels were positively associated with percent decrease in BMD whereas IL-6 levels were negatively associated. In addition, we observed significant positive correlations between baseline hs-CRP and Hp levels and percent decrease in BMD at lumbar spine as well as between hs-CRP levels and percent decrease in BMD at femoral neck. Based on Figure 1, on one hand, as the baseline levels of CRP and Hp increase the percent decrease in BMD is lower whereas when baseline levels of IL-6 increase, the percent decrease of BMD is higher. On the other hand, significant associations were noted between percent change (decrease) in physical activity energy expenditure and percent change (decrease) at lumbar spine ($r = 0.33$) and femoral neck ($r = 0.29$) BMD (all $p < 0.05$).

We then conducted stepwise multiple regression analyses to identify predictors of the individual variation of BMD in premenopausal women and percent changes in BMD observed during the 5-year follow-up. Baseline levels of hs-CRP, Hp, IL-6, cardiorespiratory fitness, physical activity energy expenditure, and body weight were used in the stepwise analysis for premenopausal women BMD variations. For percent changes in BMD over the 5-year follow-up, baseline levels of BMD (total, lumbar spine or femoral spine), hs-CRP, Hp, IL-6, and percent changes of cardiorespiratory fitness, physical activity energy expenditure, body weight and menopausal status were included based on significant correlation between these phenotypes and BMD. In premenopausal women, cardiorespiratory fitness, body weight and levels of hs-CRP explained 38%, 21% and 21% of individual variation of total, lumbar spine and femoral neck BMD,

respectively. Menopause status and baseline levels of hs-CRP, and IL-6 explained 35% of individual variations of percent change of total BMD in women transitioning to menopause. Menopause status and baseline levels of Hp explained 17% of individual variations of percent change of BMD at lumbar spine. Finally, 22% of variations in percent change of femoral neck BMD is explained by baseline hs-CRP, baseline femoral BMD and percent change of physical activity energy expenditure.

Discussion

The major finding of the present longitudinal study is the absence of association between percent changes in inflammatory markers and percent decrease in total, lumbar spine and femoral neck BMD in women transitioning to menopause. We hypothesized that inflammatory markers are associated negatively with BMD loss during the transition to menopause. However, contrary to our hypothesis, we observed positive correlations between baseline hs-CRP and Hp and percent decrease of BMD. Even if surprising, these results have been observed by other investigators. Sponholtz *et al.*⁷¹ reported a negative correlation between levels of CRP and femoral neck BMD in premenopausal women whereas in postmenopausal women using hormone replacement therapy, levels of CRP were positively associated with femoral neck BMD.⁷¹ Other cross-sectional studies have led to inconsistent results regarding the relationship between inflammatory markers and BMD.^{114,122,123} Pablo *et al.*¹²² reported a negative association between levels of CRP and total BMD in a large representative population-based sample (n=5214; age = 51±19 yr; 47% postmenopausal). This association was independent of menopause status.¹²² However, Berglundh *et al.*¹²³ demonstrated that levels of CRP were not an indicator for low BMD at femoral neck in postmenopausal women aged 75 and above. In the present study, we report negative correlations at baseline between levels of hs-CRP and BMD (total, lumbar spine, femoral neck). This opposite finding could be partly due to the significant decrease we observed in levels of hs-CRP and Hp after the 5-year follow-up. It could be that persistently elevated levels of CRP (≥ 3 mg/L) may be necessary to accelerate bone loss. In fact, Berglundh *et al.* showed that bone loss was greater among those participants with higher CRP levels (≥ 3 mg/L) sustained over 5 years.¹²³ However, that was not the case in the present study in which we observe no difference in bone loss between the subgroups with sustained high or increased levels

of hs-CRP as compared to sustained low or decreased levels of hs-CRP during the 5-year follow-up (result not shown). Although, mean CRP in our participants was lower and only 4 participants have a CRP levels ≥ 3 mg/L.

In our cohort, CRP levels were positively correlated with BMI ($r=0.41$, $p<0.05$) and body fat mass ($r=0.32$, $p<0.05$). This might partly explain why CRP is a positive predictor of BMD as higher BMI could have a protective role for BMD by transmitting more mechanical load on weight bearing skeleton.¹²⁴ Also, adipose tissue acts as an endocrine organ, producing inflammatory markers⁶. It seems that CRP could not be a good predictor of bone loss due to the complicated interaction between BMD, body fat and inflammation.

Another possibility is a difference between the actions of inflammatory markers in the bone microenvironment and their circulating levels⁷¹ or different phenotypes of inflammatory markers.¹²⁵ Duration of postmenopause is also a factor that may contribute to the relationship between inflammatory markers and BMD.⁶⁸ Ding *et al*⁶⁸ showed significantly higher negative correlations between baseline levels of CRP and IL-6, changes in levels of CRP and IL-6 and change in total BMD in women who were 10 years or more into the postmenopausal state. The short duration of the postmenopausal state of the participants of this study could partly explain why we did not observe any significant associations between changes in inflammatory markers and changes in BMD. Of note, even if 71% of our participants were transitioning to a postmenopausal status, the majority were in the early postmenopausal phase (< 5 years). Furthermore, our participants were healthy and active as compared to the general Canadian population.⁹³ Physical activity positively affects BMD via mechanical loading mechanism, increasing muscle mass and reducing inflammation.¹²⁶⁻¹²⁸ In fact, the annual rate of bone loss when participants were in perimenopausal status (0.006 g/cm² and 0.005 g/cm² for lumbar spine

and femoral neck) and in postmenopausal status (0.016g/cm² and 0.009 g/cm² for lumbar spine and femoral neck) was lower as compared to reported annual rates of bone loss for perimenopausal women (0.018 and 0.010 g/cm² for lumbar spine and femoral neck) and for postmenopausal women (0.022 and 0.013 g/cm² for the lumbar spine and femoral neck).¹²⁹

Few longitudinal studies have investigated the associations between inflammatory markers and bone loss in women. These studies mostly focused on postmenopausal women or women with hormonal replacement therapy.^{68,70} For instance, Abrahamsen *et al.*⁷⁰ reported that IL-1ra and sIL-6R were associated with the rate of bone loss in perimenopausal women. Cross-sectional studies including one performed by Gur *et al.*¹⁰⁶ revealed that IL-2, IL-8, and TNF- α may play an important role in high-turnover osteoporosis. Oppositely, Khosla *et al.*¹¹⁶ indicated that postmenopausal women with osteoporosis do not have higher levels of IL-1 α , IL-1 β , or IL-6 as compared to women with normal BMD. In line with our hypothesis, we report a negative correlation between baseline IL-6 levels and percent decrease in total BMD whereas we did not find any relationship between IL-8 and IL-1 β and BMD in our participants. Discrepancy in the results could be due to significant age gaps between cohorts (pre and postmenopausal women) in cross-sectional studies and different bone health, body composition status and lifestyle, especially diet and physical activity. Beside the opposite association observed between the baseline levels of hs-CRP and BMD, none of the inflammatory markers (IL-6, IL-8, IL-1 β , sTNFR1 and sTNFR2) were associated with BMD in our participants. This could be partly explained by the fact that higher levels of inflammatory markers have been observed in premenopausal and postmenopausal women with obesity¹³⁰ or women with inflammatory diseases such as rheumatoid arthritis¹³¹ whereas the participants of the present study were not obese (BMI = 23.31 $\pm$ 2.41 kg/m²) and healthy.

Concerning potential predictors of BMD, our results showed that hs-CRP, weight and cardiorespiratory fitness contribute to explaining the individual variations in total, lumbar spine and femoral neck BMD at baseline when all participants were in premenopausal status. Throughout the five years, menopause status, percent change of physical activity energy expenditure and baseline levels of hs-CRP, Hp, IL-6 and BMD are important predictors of percent decrease in BMD. These results further underscore the role of physical activity in attenuating BMD loss in women transitioning to menopause. Studies also demonstrated that cardiorespiratory fitness, a proxy of regular physical activity^{132,133}, positively affects BMD.¹³³ Furthermore, studies suggest physical activity is associated with lower levels of inflammatory markers.^{112,113}

We also previously reported in our MONET cohort, an increase in levels of ferritin during the 5-year follow-up.¹²¹ Unlike other studies that documented a role for ferritin in BMD loss,^{109,134} we found no significant association between levels of ferritin and changes in BMD. This could partly be because levels of ferritin seem to affect BMD during late and not early postmenopausal status.¹⁰⁸

Previously we reported that daily dietary calcium and vitamin D intake did not show any significant correlation with BMD changes through the 5 years in our participant.¹⁰² Only 6% of our participants met the Canadian Guideline recommendations for daily dietary calcium intake (1200 mg/day)¹³⁵, while none of our participants met the recommendations for daily dietary vitamin D intake (15 mcg/day or 600 IU/day)¹³⁵ throughout the 5-year study. The average intake of vitamin D was 4.1 ± 2.8 mcg/d in our cohort. Vitamin D has been shown to have an anti-inflammatory role.⁶⁴ It reduces inflammatory markers by binding to their receptors in monocytes. Also, vitamin D suppresses nuclear factor- κ B expression, which is a primary transcription factor

for inflammatory markers production.^{64,136} Furthermore, vitamin D acts as an anti-inflammatory agent by decreasing T cell responsiveness and inhibiting cellular proliferation, which reduces lymphokine production.¹³⁷ Therefore, vitamin D deficiency in our participants could partly explain the negative association between hs-CRP and bone mineral density in baseline.

Our study presents some limitations. Firstly, the population studied was composed of healthy non-obese women thus; our findings are limited to this population. However, 45% of the Canadian women aged between 40 and 59 years present a BMI between 20 and 29 kg/m².² Secondly, we did not measure levels of estradiol or estrogen, so we could not investigate the role of estrogen in the links between inflammatory markers and BMD. Also, we did not measure biomarkers of bone turn-over such as serum osteocalcin and bone-specific alkaline phosphatase. Despite these limitations, the present study enriches the scientific evidence on potential inflammatory markers predictors of BMD status in a well-characterized cohort of premenopausal women transitioning to menopause over a five-year period. We used gold standard measures methods for the measurement of BMD and body composition.¹³⁸ Also, our assessment of physical activity energy expenditure was performed by accelerometers and cardiorespiratory fitness by indirect calorimetry, which both have been shown to be valid and reliable measurements.⁸⁸

In conclusion, our results showed no significant association between change in inflammatory markers and change in BMD in women transitioning to menopause. However, baseline levels of hs-CRP, Hp, IL-6 and femoral neck BMD along with percent change in physical activity energy expenditure and menopausal status partly explained the individual variation of BMD loss in women transitioning to menopause. Additional longitudinal studies including larger sample sizes

are needed to confirm our results and the role of systemic inflammation or acting as a biomarker of bone loss in women transitioning to menopause.

Acknowledgements

Remi Rabasa-Lhoret holds the J-A DeSève research chair. We thank Beckman-Coulter (France) for the kind gift of antibodies to perform hs-CRP, orosomucoid and haptoglobin measurements.

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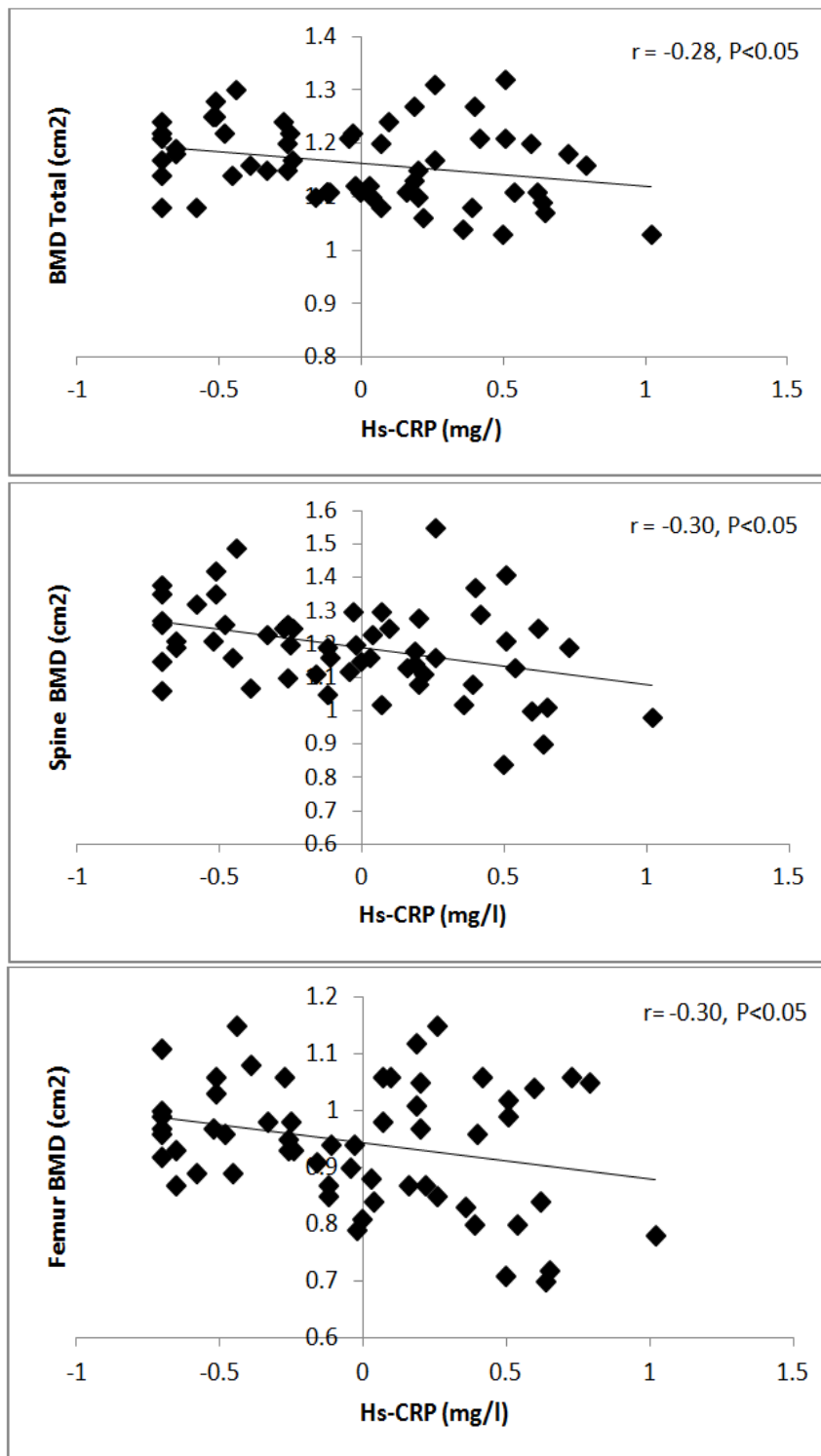


Figure 1. Partial correlation between baseline inflammatory markers and percent change of total bone mineral density (BMD) adjusted for body weight, cardiorespiratory fitness and physical activity energy expenditure; hs-CRP scores have been log transformed. After log transformation hs-CRP ranges between -0.70 to 1.02.

Table 1. Baseline characteristics of the participants

	Variable	N	Baseline
Anthropometric	Age (years)	99	49.93±1.90
	Weight (kg)	99	61.00±6.75
	Body mass index (kg/m ²)	99	23.27±2.24
	Waist circumference (cm)	99	77.89±6.72
	% Body fat	99	31.31±6.54
	Fat mass (kg)	99	19.18±5.32
	Fat-free mass (kg)	99	38.92±4.08
	<i>Vo₂ peak</i> (ml/kg/min)	95	33.83 ± 5.90
	PAEE (kcal/day)	86	805.24 ± 257.64
Inflammatory Markers	Hs-CRP (mg/l)	95	1.55±1.67
	Hp (mg/l)	94	0.87±0.33
	ApoB (g/l)	95	0.74±0.14
	Ferritin (µg/l)	85	33.32±26.57
	IL-6 (pg/ml)	85	1.03±1.01
	IL-8 (pg/ml)	85	22.78±16.22
	IL-1β (pg/ml)	85	3.81±1.89
	sTNFR1 (pg/ml)	85	1351.85±264.82
	sTNFR2 (pg/ml)	85	2689.85±400.15

Data are mean ± SD. PAEE, physical activity energy expenditure; Hs-CRP, High-sensitive C-reactive protein; ApoB, apolipoprotein B; IL-1β, interleukin 1Beta; IL-6, interleukin 6; IL-8, interleukin 8; sTNFR1 and sTNFR2, soluble tumor necrosis factor receptor 1 and 2.

Table2. Bone mineral density (g/cm²) by time point and menopausal status.

	Perimenopause (N=27)		Postmenopause (N=59)		Repeated measures ANOVA P value		
Bone mineral density(g/cm ²)	Baseline	Year 5	Baseline	Year 5	Time	status	Interaction
Total	1.16±0.07	1.15±0.06	1.17±0.07	1.14±0.07	<0.001	NS	<0.001
Lumbar spine	1.17±0.11	1.14±0.10	1.20±0.14	1.12±0.14	<0.001	NS	<0.01
Femoral neck	0.93±0.09	0.91±0.09	0.95±0.11	0.90±0.10	<0.001	NS	<0.05

Table 3. Partial correlations between inflammatory markers and bone mineral density.

Baseline	Total BMD		Lumbar spine BMD		Femoral neck BMD	
	Baseline (N=87)	% Δ (N=87)	Baseline (N=85)	% Δ (N=85)	Baseline (N=87)	% Δ (N=87)
Hs-CRP(mg/l)	-0.28*	0.37*	-0.30*	0.32*	-0.30*	0.31*
Hp (g/l)	-0.15	0.24*	-0.20	0.27*	-0.12	0.09
ApoB (g/l)	-0.18	0.23	-0.14	0.20	-0.00	0.15
Ferritin (μ g/l)	0.02	0.05	0.03	0.05	0.03	-0.09
IL-6 (pg/ml)	-0.01	-0.26*	-0.15	-0.06	0.03	-0.06
IL-8 (pg/ml)	-0.02	0.04	0.15	-0.01	-0.10	-0.05
IL-1 β (pg/ml)	-0.02	-0.08	0.08	-0.14	0.00	-0.21
sTNFR1(pg/ml)	-0.03	-0.03	0.03	0.06	-0.08	0.06
sTNFR2 (pg/ml)	-0.18	0.18	-0.13	0.21	-0.08	0.06

Δ , relative change; *, P<0.05

Hs-CRP, High-sensitive C-reactive protein; ApoB, apolipoprotein B; IL-1 β , interleukin 1Beta; IL-6, interleukin 6; IL-8, interleukin 8; ; sTNFR1 and sTNFR2, soluble tumor necrosis factor receptor 1 and 2. BMD, bone mineral density; log transformation has been done for CRP, IL-6, and ferritin. Adjusted for body weight, cardiorespiratory fitness and physical activity energy expenditure.

Table 4. Predictors of bone mineral density.

	Dependent variable	Independent variable	R	R ²	Total R ²	P value
	Baseline					
Stepwise regression	Total BMD (N=73)	Cardiorespiratory fitness	0.29	0.218	38%	0.000
		Weight	0.34	0.120		0.003
		Hs-CRP	-0.28	0.043		0.033
	Lumbar spine BMD (N=72)	Cardiorespiratory fitness	0.19	0.074	21%	0.025
		Weight	0.24	0.081		0.012
		Hs-CRP	-0.30	0.060		0.021
	Femoral neck BMD (N=73)	Cardiorespiratory fitness	0.23	0.079	21%	0.010
		Weight	0.26	0.069		0.021
		Hs-CRP	-0.30	0.068		0.026
	%Δ BMD					
Stepwise regression	Total BMD (N=73)	Menopause Status	-0.44	0.198	35%	0.000
		Baseline Hs-CRP	0.37	0.075		0.006
		Baseline IL-6	-0.26	0.076		0.009
	Lumbar spine BMD (N=72)	Menopause Status	-0.33	0.049	17%	0.003
		Baseline Hp	0.27	0.123		0.048
	Femoral neck BMD (N=73)	Baseline Hs-CRP	0.31	0.110	22%	0.004
		%ΔPhysical Activity Energy Expenditure	0.28	0.051		0.043
		Baseline femoral neck BMD	-0.27	0.066		0.017

Hs-CRP, High-sensitive C-reactive protein; BMD, bone mineral density; % Δ , percentage change.

For baseline bone mineral density, stepwise regression analysis included baseline CRP, Hp, IL-6, physical activity energy expenditure, cardiorespiratory fitness and weight whereas for percent change in bone mineral density during the menopause transition, menopause status at year 5, baseline CRP, Hp, IL-6, baseline bone mineral density, percent change of physical activity energy expenditure, cardiorespiratory fitness and weight were used.

CHAPTER 6

BODY COMPOSITION, CARDIOMETABOLIC RISK FACTORS, PHYSICAL ACTIVITY,
AND INFLAMMATORY MARKERS IN PREMENOPAUSAL WOMEN AFTER A 10 YEAR
FOLLOW UP: A MONET STUDY

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(Menopause. 2017 Accepted)

Author contributions:

Éric Doucet, Rémi Rabasa-Lhoret, Martin Brochu, Jean-Marc Lavoie, and Denis Prud'homme participated in the development of the research project (MONET). Sahar Razmjou and Joseph Abdounour participated in the data collection. Jean-Philippe Bastard and Soraya Fellahi participated in the blood analysis and inflammatory makers measurements. Sahar Razmjou and Denis Prud'homme performed the analysis and interpretation and completed the writing of the manuscript. All authors were involved in the revision and interpretation of the paper.

Body Composition, Cardiometabolic Risk Factors, Physical Activity, and Inflammatory Markers in Premenopausal Women after a 10 year follow-up: A MONET Study

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Running title: Post-menopause and inflammatory markers

Conflicts of interest: None reported.

Funding/support: This research was supported by a grant received by Denis Prud'homme from the Canadian Institutes of Health Research (CIHR) (T 0602145.02).

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Abstract

Objective: The menopause transition and postmenopausal periods are usually associated with changes in body composition and a decrease in physical activity energy expenditure. This study investigated body composition, cardiometabolic risk factors, physical activity energy expenditure (PAEE), and inflammatory makers in premenopausal women after a 10-year follow-up.

Design/Methods: One hundred and two premenopausal women participated in the 5-year observational longitudinal Montreal Ottawa New Emerging Team (MONET) study. This present sub-study included 48 participants (age: 60.0 ± 1.7 years; body mass index: 23.2 ± 2.2 kg/m²) 6.0 $\pm$ 0.3 years after completion of the initial MONET study. Measures included body composition, waist circumference (WC), fasting glucose and insulin levels, insulin sensitivity (QUICKI model), plasma lipid levels, PAEE and inflammatory markers.

Results: Compared to baseline measures of the MONET study, analyses revealed no significant increase in body weight although there were significant increases in WC, fat mass (FM), % FM, total cholesterol (TC), low (LDL-C) and high density lipoproteins (HDL-C), haptoglobin (Hp), Apolipoprotein B (ApoB), ferritin, adiponectin, and soluble cluster of differentiation 14 (sCD14) (all $p < 0.001$) after the 10-year follow-up. However, significant decreases were observed for fat free mass (FFM), PAEE, fasting glucose levels, interleukin 8 (IL-8) levels and soluble tumor necrosis factor receptor 1 and 2 (sTNFR-1 and sTNFR-2) levels (all $p < 0.05$). To determine the effect of postmenopausal years, data was restructured based on final menstrual period (FMP) and one-way ANOVAs were performed. Results showed that WC, % FM, TC, HDL-C, ApoB, ferritin, adiponectin, sCD14 were higher in early and late postmenopausal periods in these women. sTNFR-1 and sTNFR-2 levels were higher at the FMP and early postmenopausal years

as compared to the late postmenopausal periods. Finally, IL-8 levels were lower in years after FMP. **Conclusion:** The number of years elapsed since the FMP can affect body composition, cardiometabolic risk factors and inflammatory markers in healthy premenopausal women going through menopause transition and postmenopausal periods.

Key words: Postmenopause, inflammatory markers, lipid profile, body composition, physical activity, cardiometabolic risk factors

Introduction

The menopause transition is associated with an increased risk of developing the metabolic syndrome and cardiovascular diseases¹³⁹. There is a growing body of evidence that chronic inflammation is a contributing factor to cardiometabolic alterations associated with menopause.¹⁴⁰ Previous studies have shown that inflammatory markers such as C-reactive protein (CRP), ferritin, interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF- α) are associated and could play a role in the development of cardiometabolic risk factors.^{140,141} Furthermore, the level of these inflammatory markers is known to be higher in postmenopausal women.^{6,9,20,30,33} However, studies investigating inflammatory markers and menopause have mainly focused on aging and comparing different groups of pre and postmenopausal women rather than investigating the effect of the number of years since the final menstrual period (FMP).

Menopause-related body composition changes, especially increases in fat mass (FM) and visceral fat, and plasma lipid alterations contribute to increasing the risk of cardiovascular diseases.^{142,143} In fact, visceral fat constitutes 5-8% of total FM in premenopausal women which has been shown to increase up to 15-20% in postmenopausal women.¹⁴⁴ Visceral fat acts as an endocrine organ secreting inflammatory markers that play a role in the development of the metabolic syndrome and cardiovascular diseases.¹⁴⁵

Epidemiological studies indicate that premenopausal women have a lower incidence of cardiometabolic disease as compared to postmenopausal women.^{32,146,147} Therefore, postmenopausal status (years since the FMP) could play an important role in the prevalence of the cardiometabolic diseases. Since it is difficult to distinguish the difference between the effects

of natural aging from those of menopause, few studies^{23,54,55,148,149} have addressed the influence of the duration (in years) since the FMP on body composition and cardiometabolic risk factors in healthy normal weight women.

Numerous studies^{23,54,148,149} have shown that postmenopausal women are at a higher risk of developing abdominal obesity and the metabolic syndrome. They also have a higher body mass index (BMI), waist circumference (WC), blood pressure, CRP, cholesterol, triglyceride and low density lipoprotein cholesterol (LDL-C) levels whereas the direction of change of blood glucose and high lipoprotein cholesterol (HDL-C) is not consistent among studies. In addition, Wang *et al*⁵⁵ showed in postmenopausal women that fat free mass (FFM) decreased with years since menopause while FM and body fat distribution were not related to years since menopause but with age.

Decreased level of physical activity energy expenditure (PAEE) and its association with body composition alterations in postmenopausal women have been reported previously.¹⁵⁰⁻¹⁵² However these studies accounted for age but not for years since the FMP. These studies showed that the level of physical activity decreases in midlife and older women. However, those who maintained or increased their physical activity level tended to experience less change in body composition.¹⁵⁰⁻¹⁵²

None of these longitudinal studies reported inflammatory markers and/or PAEE changes and their association with body composition and cardiometabolic risk factor changes using gold standard techniques over an extensive postmenopausal period in healthy women. Documenting the changes of body composition over time and cardiometabolic risk factor alterations may contribute to identifying timely preventive interventions that could decrease the risk of developing the metabolic syndrome and/or cardiometabolic diseases in postmenopausal women.

This study will investigate how body composition, cardiometabolic risk factors, PAEE and the inflammatory markers change after a 10-year follow-up in healthy normal weight premenopausal women. We hypothesized women will have an increase in FM, WC, inflammatory markers and a decrease in FFM, and PAEE. Furthermore, we also aimed to assess how the duration (number of years) since the FMP affects these primary outcomes.

Methods

The present article is a sub-study of the Montreal Ottawa New Emerging Team (MONET) study. The MONET study consisted in a 5-year longitudinal study (2004 to 2009) on the effects of the menopause transition on body composition and cardiometabolic risk factors in 48 healthy premenopausal women aged between 47 and 55 years. The complete design and methodology of the MONET study has been described previously.² The present sub-study is an observational community based study which consisted in contacting - by sending out letters - participants after completion of the MONET study for a series of follow-up assessments. Figure 1 presents the recruitment diagram and participants sample size. Inclusion criteria for the present study were the following: healthy women that participated and completed the MONET study. Exclusion criteria includes: (1) medical problems or medication that could interfere with outcome variables such as Cushing's disease, polycystic ovary syndrome, thyroid gland disease or cancer; (2) a history of drug and/or alcohol abuse; (3) medications including antipsychotics/antidepressants, oral corticosteroids or anti-obesity medications. Three and ten women were receiving hormone replacement therapy at the 5-year and 10-year follow-up, respectively. One woman had undergone a hysterectomy and three women had removed one ovary. Women were instructed not to restrict diet and/or physical activity. (4) $BMI \geq 30 kg/m^2$. The study was approved by the University of Ottawa and the Montfort Hospital Ethics Committees. All participants signed a written consent form prior to their participation in the study.

Study Design- Participants were invited to the laboratory for the following tests and measures:

1) anthropometric and body composition (dual-energy x-ray absorptiometry [DXA]) measurements; and 2) fasting blood samples for the measurement of fasting plasma lipid, insulin, glucose, and inflammatory marker levels. Menopause status was determined using a self-

reported questionnaire about menstrual bleeding and its regularity. Women were classified as premenopausal if they reported no change in menstrual cycle frequencies and perimenopausal if they reported changes in menstrual frequency and/or amenorrhea for 3 to 11 months. Finally, women were classified as postmenopausal based on their FMP and confirmed by 12 months of amenorrhea.²

Anthropometrics- Body weight and height were measured using a BWB- 800AS digital scale and a Tanita HR-100 stadiometer, respectively (Tanita Corporation of America Inc., Arlington Heights, IL). BMI was calculated as body weight in kilograms divided by height in square meters (kg/m^2). WC (mean of two measurements) was determined using a Gulick tape at the middle distance between the lowest rib and the iliac crest.^{2,82} FM, % FM, and FFM were measured using dual-energy x-ray absorptiometry (GE-LUNAR Prodigy module; GE Medical Systems, Madison, WI) as previously described.^{2,83} Using duplicate measurements of % FM in 12 healthy participants tested in our laboratory, the coefficient of variation and the correlation for the reproducibility were 1.8% and $r = 0.99$, respectively.

Physical activity energy expenditure- An Actical accelerometer (Mini Mitter Co., Inc., Bend, OR) worn on the right hip (anterior to the iliac crest) and secured with an elastic belt with the arrow pointing up was used to measure daily physical activity and to estimate mean PAEE. During 7 days following the lab visit, participants wore the accelerometer upon waking up and took it off just before going to bed (90% reliability for the measurement of physical activity⁸⁷). The accelerometers used in this study were validated previously with the use of doubly labeled water measurements to estimate physical activity energy expenditure.⁸⁸

Blood sampling- Blood samples were taken after a 12-hour overnight fast. Plasma glucose levels were determined using spectrophotometric analysis after conversion of glucose to glucose-6-phosphate by hexokinase (Sigma-Aldrich Canada Ltd., Oakville, Ontario, Canada; Fisher Scientific Limited, Nepean, Ontario, Canada). Plasma insulin concentrations were determined by radioimmunoassay using ^{125}I -labeled human insulin and a human insulin antiserum (Millipore, St. Charles, MO). Insulin resistance was estimated using the QUICKI model (score) by the following equation: $1/[\log \text{ insulin (mU/l)} + \log \text{ glucose (mg/dl)}]$.⁸⁹

Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) levels were analysed using the Vitros 950 immunoassay analyzer (Ortho Clinical Diagnostics; Johnson & Johnson Company, Markham, Ontario, Canada) at a wavelength of 540 nm. TC, HDL-C, and TG were used in the Friedwald formula to calculate low-density lipoprotein cholesterol (LDL-C) concentrations.¹⁵³

For CRP, orosomucoid (ORM) and haptoglobin (Hp), assessments were made by immunonephelometry using an image analyzer (Beckman-Coulter, Villepinte, France) with detection limits of 0.20 mg/L, 0.25 g/L and 0.08 g/L respectively. Apolipoprotein B (apoB) was assessed by immunoturbidimetry (Architect, Abbott, Rungis, France) with detection limits of 0.03 g/L. Serum ferritin concentration was measured by chemiluminescence (Architect, Abbott, Rungis, France) with detection limits of 1 ng/mL. Serum high sensitivity IL-6, IL-8, soluble tumor necrosis factor receptor 1 and 2 (sTNFR-1 and sTNFR-2), soluble cluster of differentiation 14 (sCD14) and adiponectin levels were measured using standard and high sensitivity commercial enzyme-linked immunosorbent assay (ELISA) kits (Quantikine®, Abingdon, UK) with detection limits of 0.04 pg/mL, 3.50 pg/mL, 0.77 pg/mL, 0.60 pg/mL, 125 pg/mL, and 0.25

ng/mL, respectively. Coefficients of variation for inter- and intra-assay reproducibility were < 10% for biomarker measurements performed with ELISA (IL-6, IL-8, sTNFR-1, sTNFR-2, sCD14 and adiponectin) and < 5% for other inflammatory markers, namely ferritin, ORM, Hp, ApoB and CRP.

Statistical analysis-Descriptive results are expressed as the mean $\pm$ standard deviation. Normality was determined and then CRP, ferritin, IL-6 and IL-8 were log-transformed to allow normality of distribution. Repeated-measure analysis of variance (ANOVA)s were performed to determine changes in variables of interest from premenopausal status to postmenopausal status.

A one-way ANOVA was performed to compare the difference of variable changes between years relative to FMP. We re-organized the data in cases and divided participants into 5 groups based on their menopausal status at baseline (all premenopausal), at year five [2% premenopausal (n=1), 35% perimenopausal (n=17) and 60% postmenopausal (n=29)], and after 10 years (all postmenopausal); Group 1: FMP -9 to -5, group 2: -5 to -1, group 3: FMP 0, group 4: FMP +1 to +5, and group 5: FMP +5 to +9. Year 0 is the year within the FMP; year 1 is considered as 1 year after FMP, year -1 is considered as 1 year before FMP, and so on. For example, if a participant was at -5FMP at the beginning of the study, it means she had her first menopausal year 5 years after she was recruited in the study and she has been postmenopausal for 5 year at the time of analysis. Pearson correlation was performed to investigate the relationship between variables. We also used mixed model linear analysis to confirm our results. SPSS 16.0 for windows (SPSS Inc., Chicago, IL) was used to perform statistical analyses. A p-value less than 0.05 was considered statistically significant.

Results

At baseline, all women were premenopausal. By the end of year five, 2% (n=1) were still premenopausal, 35% (n=17) perimenopausal and 60% (n=29) were postmenopausal. At the 10-year follow-up (average of 10.1 ± 0.3 yrs), all women were postmenopausal.

In Table 1, significant effects of time were noted with increases for WC, FM and % FM (all $p < 0.001$) and decreases for FFM despite the absence of significant differences in body weight and BMI. Furthermore, a significant effect of time was observed with increases for TC, HDL-C, LDL-C, while decreases were observed for glucose levels, PAEE, resting systolic and diastolic blood pressure ($p = 0.001-0.05$) (Table 2). We performed additional analysis with and without the participants under hormone replacement therapy and the results were the same (data not shown).

As presented in Table 3, levels of Hp ($P < 0.05$), ApoB, ferritin, adiponectin, sCD14, IL-8, sTNFR-1, and sTNFR-2 (all $p < 0.001$) increased at follow-up while IL-8, sTNFR-1 and sTNFR-2 levels decreased.

Effect size for body composition variables with significant change was between 0.22-0.50 with an observed power of 0.94-1.00. Effect size for cardiometabolic variables with significant change was between 0.12-0.65 with an observed power of 0.69-1.00. Effect size for inflammatory markers with significant change was between 0.11-0.59 with an observed power of 0.67-1.00.

As shown in Table 4, WC was gradually higher from years prior to the FMP to the years after final menstrual period. Percentage FM was significantly higher in +1 to +5 FMP and +5 to +9

FMP groups as compared to -9 to -5 FMP group. Moreover, levels of TC and HDL-C were gradually higher from years prior to the FMP to the years after FM after FMP. PAEE was lower in +5 to +9 FMP group as compared with 0 FMP and -5 to -1 FMP groups. Finally, resting diastolic blood pressure was lower in +5 to +9 FMP group as compared with all other FMP groups (Table 5).

Finally, ApoB, ferritin, sCD14, and adiponectin levels were significantly higher while IL-8 levels were gradually lower years prior to the FMP as compared to years after FMP (Figure 2). However, sTNFR-1 and sTNFR-2 levels were higher in -5 to -1 FMP and 0 FMP groups as compared with +5 to +9 FMP group. We also used mixed model linear analysis from which we obtained the same results except for adiponectin and fat mass that were no longer significant.

Significant negative correlation was found between years since FMP and % change in PAEE ($r = -0.38$, $P < 0.05$).

Discussion

To our knowledge, this is the first longitudinal study documenting inflammatory markers and PAEE changes over an extensive postmenopausal period in healthy non obese women. One major finding of the present study is that we observed no increase in body weight and BMI in our healthy participants after an average of six years postmenopause. As previously reported² in the original MONET study, we observed an increase in FM, % FM and a decrease in FFM with time. However, body fat distribution changes become more evident as years since FMP progressed. These results support the concept that menopause transition and postmenopausal periods affect body fat distribution and body composition and not body weight per se. Our results are consistent with studies that reported a role of menopause transition and of the postmenopausal period in body composition and body fat distribution change.^{148,154} However, they are in contradiction with other studies that found aging to be more important regardless of menopausal status.^{151,155} One study used a cross sectional design¹⁵⁵ and in the other study¹⁵¹, the follow-up periods was shorter (3 years).

The changes in body composition observed in our participants could be partly related to the reduction of PAEE due to a shift to a more sedentary lifestyle after menopause, as demonstrated by a significant negative correlation between years since FMP and % change in PAEE ($r = -0.38$, $P < 0.05$). In fact, our participants decreased their PAEE by 17% and 23.2% since premenopausal status and FMP, respectively. Another factor that may play a role in menopause related changes in body composition and body fat distribution is the progressive decline of estrogen levels. As reported by other researchers, estrogen deficiency mediates changes in body fat distribution and body composition by increasing FM especially visceral fat and decreasing FFM.^{156,157}

Our results showed significant increases in TC and LDL-C. These findings support the hypothesis that the increase in cardiovascular diseases in postmenopausal women could be, in part, related to the progressive increase in LDL-C with aging and the increase in TC after menopause.⁵⁴

Although it is known that HDL-C is normally lower in postmenopausal women¹⁵⁸, to our surprise values increased progressively with higher levels than baseline in our participants. This HDL-C trajectory has also been previously reported.^{159,160} One possible explanation for the increase in HDL-C levels could be the level of PAEE. It is well documented that high physical activity levels are associated with higher HDL-C levels.^{161,162} Although we demonstrated that level of PAEE decreased in our participants, they are still on average, more active (average 242 minutes moderate and 29 minutes vigorous physical activity per week) than guideline recommendations (150 minutes of moderate to vigorous intensity physical activity/ week).⁸² According to Canadian Health Measures Survey (CHMS) data, only 5% of Canadian women aged 40 to 59 year meet the recommended guideline.¹⁶³ Higher level of physical activity in our women, could also partly explain the reduction observed in glucose levels and diastolic blood pressure with time and menopausal status.¹⁶⁴

A second major finding of the present study is that inflammatory markers including Hp, ApoB,¹⁶⁵ ferritin, sCD14, and adiponectin levels increased while sTNFR-1, sTNFR-2, and IL-8 levels decreased at 10-year follow-up. The increase in inflammatory markers could be due to the shift in fat deposition that accompanies menopause, which normally leads to more visceral fat deposition, which acts as an endocrine organ producing inflammatory markers.^{78,145} Considering that we also observed a progressive increase in WC during the postmenopausal years in our

women (an average of 6.48 ± 6.49 cm in 10 years), we performed another analysis to investigate the role of menopause and postmenopausal periods. Results showed that levels of ApoB, Ferritin, sCD14, and adiponectin increased significantly years prior to the FMP until years after FMP. Levels of sTNFR-1 and sTNFR-2 were significantly higher around the FMP and early postmenopausal years as compared to late postmenopausal years. These results are consistent with a previously published cross sectional study¹⁶⁶ that reported levels of TNF- α were higher during the early postmenopausal period (6.2 years since menopause) as compared to pre and peri menopausal women. However, our results are in contradiction with another cross sectional study²⁶ that showed levels of TNF- α were lower in postmenopausal women (age 54 ± 8 years, range 48–63) compared with premenopausal women (age 32 ± 7 years, range 18–45). These studies did not report the years since FMP or body composition characteristics of the women. Large age gaps (from 30 to 60 years) between cohorts (premenopausal and postmenopausal women) or other factors such as differences in body composition or use of hormone therapy between study populations could explain discrepancies among the findings.

Increased concentrations of sCD14, a protein expressed in hepatocytes and adipocytes that mediates the proinflammatory signal¹⁶⁷, have been related to cardiometabolic disorders and cardiovascular diseases.¹⁶⁸ Our results showed that sCD14 levels increased significantly from baseline to 10 years notably during the early and late postmenopausal periods. In a study by Reiner *et al*¹⁶⁹, the authors demonstrated strong positive correlations between sCD14 with measures of vascular diseases such as carotid wall thickness. In our population, ferritin levels increased from baseline to year 10. In the additional analysis, level of ferritin increased significantly from years prior to the FMP to the years after the FMP. As the menstrual cycle stops, serum ferritin increases two to threefold because of the absence of vaginal bleeding. This

has been confirmed by other studies.^{91,99} Similarly, Kim *et al.* found an increase in ferritin levels with menopause.⁹⁹ However, contrary to what we expected, adiponectin levels increased in this sub-study. Previous studies have shown that body fat distribution is related to adiponectin level and increased visceral fat is associated with low levels of adiponectin secretion while subcutaneous fat is not.¹⁷⁰⁻¹⁷² It is possible that increase in WC observed in our participants is due to a greater accumulation of subcutaneous fat. Also, physical activity has been shown to increase serum adiponectin¹⁷³ and our participants were on average, physically active. Furthermore, we did not measure high molecular weight adiponectin, which has been suggested to be a better predictor of metabolic parameters than total adiponectin.¹⁷⁴ This could account for the lack of consistency. These results have been reported by other investigators.⁵⁸ Furthermore, our results showed IL-8 decreases significantly from baseline to year 10. This has been also previously reported by another group.¹⁷⁵

The findings of our study should be interpreted considering certain limitations. Firstly, the population studied was composed of healthy women within a narrow range of BMI. Therefore, our results are limited to this subgroup of the population. Still, it is notable to point out that 45% of the women aged between 40 and 59 years in the Canadian population present a BMI between 20 and 29 kg/m². Secondly, the number of participants in the study decreased throughout the study, which reduces statistical power. Finally, we used the more cost-efficient index (waist circumference) to estimate abdominal fat instead of measuring visceral fat area by computed tomography.

Despite these limitations, the present study consists of a well-characterized cohort of women that were followed for 10 years. The longitudinal design allowed us to compare the same women as

they went through the menopause transition and postmenopausal years, and thus limit intra-individual variation in factors that could account for the effects on inflammatory markers. Lastly, we used gold-standard methods for the measurement of body composition (dual-energy x-ray absorptiometry) and daily PAEE (accelerometer).

Conclusion

This longitudinal 10-year follow-up study indicates that the postmenopausal period is associated with a decrease in physical activity and glucose levels as well as an increase in waist circumference, % FM and total cholesterol and HDL levels. Levels of inflammatory markers (including ApoB, ferritin, adiponectin, sCD14) were higher in early and late postmenopausal periods while sTNFR-1 and sTNFR-2 levels were higher around the final menstruation period and early postmenopausal years. Finally, we observed being physically active could lead to an increase of some biomarkers potentially "beneficial" (adiponectin and HDL-C) in healthy premenopausal women going through menopause transition and postmenopausal periods. However, it is possible that the increase in low-grade inflammation and lipid disorders that we observed, may outweigh the beneficial effects and may, in the long term, overcome them.

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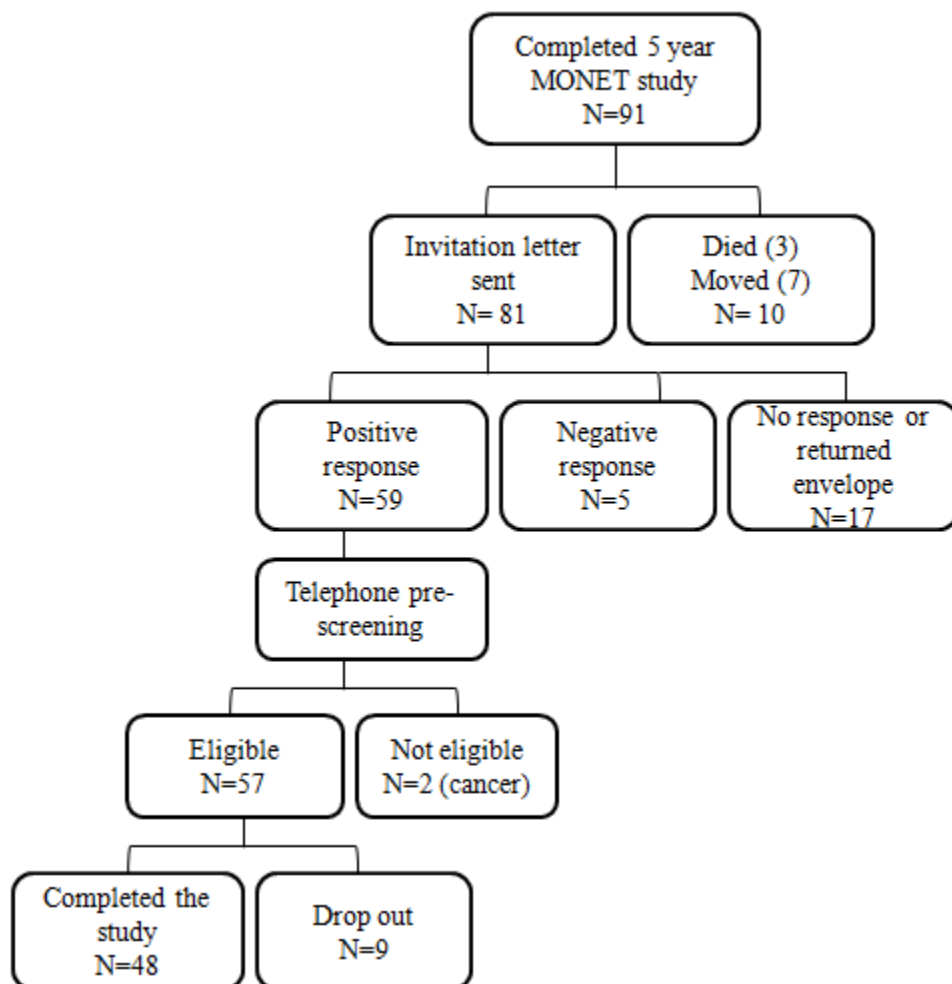


Figure 1. Recruitment process and sample size of women.

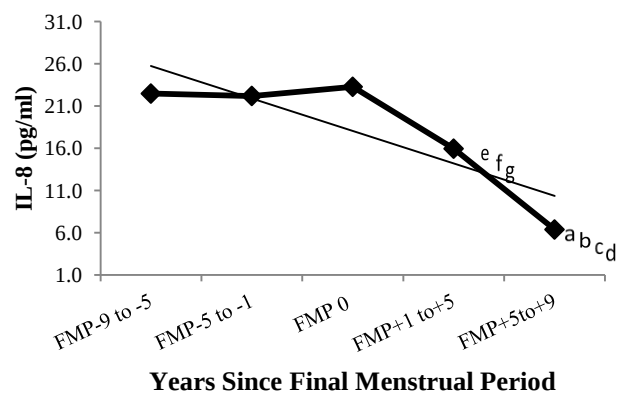
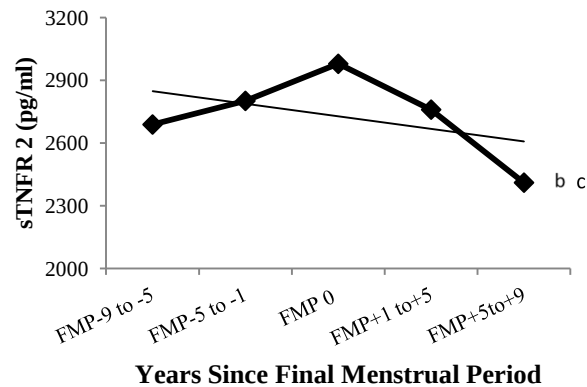
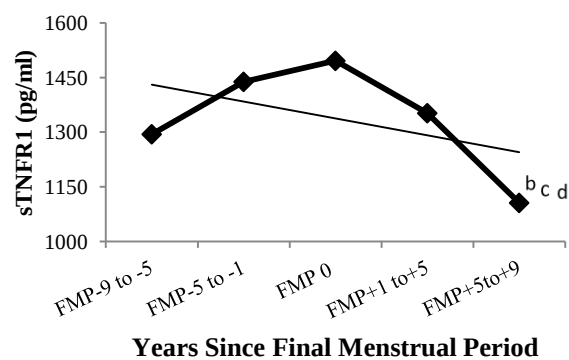
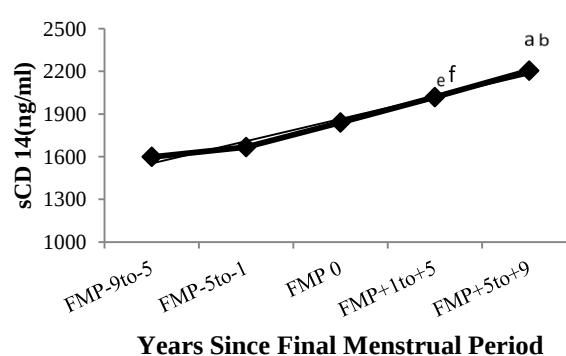
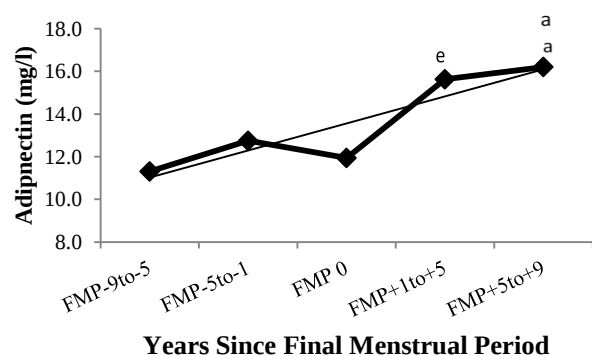
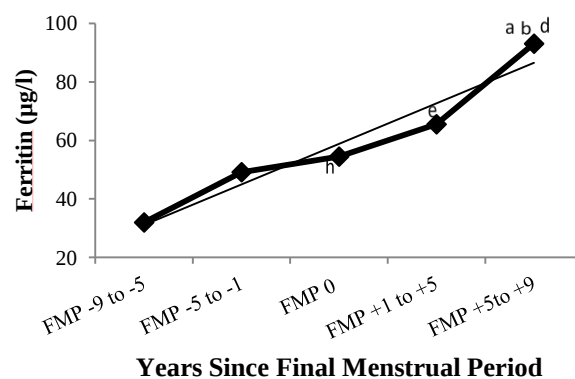
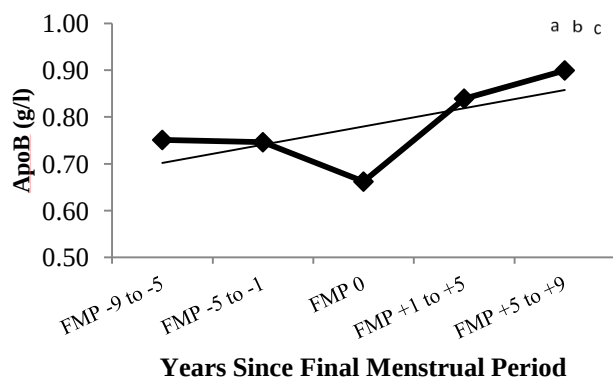


Figure 2. Inflammatory markers changes since final menstrual period (FMP) in 5 groups [group 1: FMP -9 to -5, group 2: -5 to 0, group 3: 0 FMP , group 4: FMP +1 to +5, group 5 :FMP +5 to +9] a, significant difference between G1 and G5; b, significant difference between G2 and G5; c, significant difference between G3 and G5; d, significant difference between G4 and G5; e, significant difference between G1 and G4; f, significant difference between G2 and G4; g, significant difference between G3 and G4; h, significant difference between G1 and G3. Thin lines are trend lines.

Table 1. Body composition characteristics of the participants by time point.

	Baseline	10 Years (10.12±0.33)	ANOVA-Repeated Measure P-value
N	48		
Age (yrs)	49.77±1.80	59.97±1.78	P<0.001
Weight (kg)	60.89±6.61	62.04±8.20	NS
BMI(kg/m ²)	23.24±2.27	23.79±2.76	NS
WC (cm)	78.04±7.02	84.56±7.60	P<0.001*
FM (%)	30.83±6.83	34.10±7.94	P<0.001*
FM (kg)	18.88±5.37	21.38±7.07	P<0.001*
FFM (kg)	39.17±4.20	37.96±4.05	P<0.001*

BMI, body mass index; WC, waist circumference; FM%, percent fat mass; FM, fat mass, FFM, fat free mass; NS, not significant; .

Table 2. Cardiometabolic characteristics of the participants by time point.

	Baseline	10 Years (10.12±0.33)	ANOVA-Repeated Measure P-value
N	48		
TG (mmol/l)	0.84±0.30	0.95±0.44	NS
TC (mmol/L)	4.49±0.63	5.20±0.66	P<0.001*
LDL-C (mmol/L)	2.52±0.60	2.81±0.66	P<0.05*
HDL-C (mmol/L)	1.58±0.37	1.96±0.51	P<0.001*
TC/HDL-C ratio	2.98±0.74	2.83±0.84	NS
Glucose (mmol/L)	4.78±0.32	4.46±0.47	P<0.001*
Insulin (μU/ml)	11.56±3.00	11.42±7.62	NS
QUICKI	0.34±0.03	0.34±0.02	NS
PAEE (kcal/day)	838.97±291.61	698.13±243.55	P<0.05*
SBP(mm Hg)	117.60±12.34	112.26±14.40	P<0.05*
DBP (mm Hg)	73.27±8.03	69.37±8.06	P<0.05*

TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; PAEE, physical activity energy expenditure; SBP, systolic blood pressure, DBP, diastolic blood pressure; NS, not significant. .

Table 3. Inflammatory markers of the participants by time point.

	Baseline	10 Years	ANOVA-Repeated Measure P-value
N	48		
Hs-CRP (mg/l)	1.51±1.86	1.85±3.67	NS
ORM (g/l)	0.63±0.13	0.66±0.17	NS
Hp (g/l)	0.86±0.35	0.96±0.41	P<0.05*
ApoB (g/l)	0.75±0.14	0.91±0.17	P<0.001*
Ferritin (μg/l)	32.38±23.67	86.23±59.93	P<0.001*
Adiponectin (mg/l)	12.29±4.70	15.74±6.58	P<0.001*
sCD14(ng/ml)	1653.07±306.69	2235.73±398.27	P<0.001*
IL-6 (pg/ml)	0.99±0.50	1.16±2.30	NS
IL-8 (pg/ml)	25.58±20.97	6.49±4.78	P<0.001*
sTNFR-1(pg/ml)	1364.22±280.62	1108.46±173.64	P<0.001*
sTNFR-2 (pg/ml)	2721.47±396.28	2424.09±508.35	P<0.001*

Values are mean ± SD. CRP, high sensitive c-reactive protein; ORM, orosomucoid; Hp, haptoglobin; ApoB, apolipoprotein B; IL-6, Interleukin 6; IL-8, Interleukin 8; sTNFR-1,2, Soluble tumor necrosis factor receptor 1 and 2. Log transformation scores for CRP, Ferritin, IL-6 and IL-8 have been used for analysis but physiological values have been reported. NS, not significant;

Table 4. Body composition changes of participants since final menstrual period.

Groups	Years Since Final Menstrual Period					ONE WAY ANOVA P-value
	G1 -9 to -5 FMP	G2 -5 to -1 FMP	G3 0	G4 +1to +5 FMP	G5 +5 to +9 FMP	
N	25	38	9	38	32	
Weight (kg)	60.10±6.88	61.79±6.55	59.23±7.47	62.42±7.40	61.72±8.39	NS
BMI(kg/m ²)	22.99±2.10	23.56±2.42	22.91±2.14	23.82±2.88	23.69±2.80	NS
WC (cm)	76.88±7.64	78.36±6.60	76.32±4.50	81.15±7.14	85.26±7.21	P<0.001a,b,c,d,e
FM (%)	29.26±6.25	32.55±7.26	29.85±7.31	34.68±7.53	34.13±8.16	P<0.05a,e
FM (kg)	17.68±5.02	20.22±5.81	17.86±6.16	21.81±6.55	21.33±7.32	NS
FFM (kg)	39.70±4.48	38.71±4.09	38.71±4.04	37.82±3.86	37.70±3.83	NS

BMI, body mass index; WC, waist circumference; FM%, percent fat mass; FM, fat mass; FFM, fat free mass; final menstrual period (FMP); [group 1: FMP -9 to -5, group 2: -5 to 0, group 3: 0 FMP, group 4: FMP +1 to +5, group 5 :FMP +5 to +9]; NS, not significant; a, significant difference between G1 and G5; b, significant difference between G2 and G5; c, significant difference between G3 and G5; d, significant difference between G4 and G5; e, significant difference between G1 and G4

Table 5. Metabolic and Physiologic characteristics changes since final menstrual period.

Groups	Years Since Final Menstrual Period					ONE WAY ANOVA P- value
	G1 -9 to -5 FMP	G2 -5 to -1 FMP	G3 0	G4 +1to +5 FMP	G5 +5 to +9 FMP	
N	25	38	9	38	32	
TG (mmol/l)	0.84±0.37	0.87±0.31	0.87±0.36	0.92±0.42	0.90±0.37	NS
TC (mmol/L)	4.38±0.64	4.69±0.67	4.82±0.48	5.19±0.72	5.13±0.62	P<0.001a,b,e,f
LDL-C (mmol/L)	2.47±0.59	2.63±0.63	2.66±0.46	2.92±0.66	2.77±0.64	NS
HDL-C (mmol/L)	1.53±0.36	1.66±0.39	1.76±0.34	1.85±0.45	1.97±0.48	P<0.05a,b,e
TC/HDL-C ratio	2.99±0.72	2.95±0.73	2.79±0.50	2.96±0.78	2.75±0.78	NS
Glucose (mmol/L)	4.81±0.31	4.70±0.33	4.52±0.17	4.49±0.33	4.41±0.51	P<0.05a,b,e,f
Insulin (μUm/L)	11.53±3.38	10.91±2.97	9.62±3.28	11.09±5.86	11.61±7.57	NS
QUICKI	0.34±0.04	0.34±0.03	0.34±0.01	0.34±0.02	0.34±0.01	NS
PAEE (kcal/day)	779.54±221.75	879.06±297.89	900.77±169.10	764.63±290.28	691.23±229.24	P<0.05b,c
Resting SBP(mm Hg)	116.24±12.15	119.42±11.39	119.50±12.03	116.26±11.41	112.03±15.47	NS
Resting DBP (mm Hg)	72.44±8.07	75.35±7.78	74.50±6.99	72.66±7.13	68.06±7.46	P<0.05a,b,c,d

TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; PAEE, physical activity energy expenditure; SBP, systolic blood pressure, DBP, diastolic blood pressure, final menstrual period (FMP); [group 1: FMP -9 to -5, group 2: -5 to 0, group 3: 0 FMP , group 4: FMP +1 to +5, group 5 :FMP +5 to +9]; NS, not significant; a, significant difference between G1 and G5; b, significant difference between G2 and G5; c, significant difference between G3 and G5; d, significant difference between G4 and G5; e, significant difference between G1 and G4; f, significant difference between G2 and G4

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSION

The prevalence of chronic diseases, including cardiovascular heart disease, metabolic syndrome and osteoporosis, increases during menopause and postmenopausal years.¹⁷⁶ Menopause may trigger a cascade of deleterious metabolic events. Indeed, the prevalence of metabolic syndrome is reported to range between 13.7 to 41.5% in postmenopausal women.¹⁷⁷ Menopause-related body composition changes, especially increases in fat mass and visceral fat, and plasma lipid alterations contribute to increasing the risk of cardiovascular diseases.¹⁷⁶ Increase in visceral fat, may contribute to the development of a pro-inflammatory state.⁶ Pro-inflammatory markers are important risk factors for the development of insulin resistance, metabolic syndrome, diabetes and cardiovascular diseases.¹⁷⁸ There has been little longitudinal work directed at changes in inflammatory markers and/or physical activity energy expenditure and its association with body composition and cardiometabolic risk factors changes using gold standard techniques during menopause transition and over an extensive postmenopausal period in healthy non obese women. The Montreal-Ottawa New Emerging Team (MONET) study was conducted to enhance the knowledge in this regard. MONET study was a 5 year longitudinal observational study that investigates changes in body composition and cardiometabolic risk factors in none obese women going through the menopause transition. The study included 102 premenopausal women between the age of 47 and 55 years at baseline. The primary objective of the present thesis was to examine the effect of menopause transition on inflammatory markers, by accounting for body composition and physical activity changes in healthy non-obese women going through the menopause transition. Although participants on average did not gain body weight throughout the

5-year follow-up into the menopause transition, they did increase their body fat mass and waist circumference, suggesting that menopause could have more influence on body composition and fat distribution.¹⁵⁷ Also, menopausal transition is accompanied by an increase in inflammatory markers, namely ferritin, IL-8, and sTNFR 1 and 2. The increase in inflammatory markers with menopause could be explained, in part, by changes in fat mass and peripheral fat, respectively. In fact, we observed positive correlations between body composition indices and inflammatory markers. The strongest positive associations were observed between changes in waist circumference and peripheral fat and changes in inflammatory markers (ORM, ApoB).

Transition from premenopause to postmenopause is associated with increased fat mass and central fat, especially the accumulation of visceral abdominal fat.^{2,6} With increased fat mass and adipocyte hypertrophy, the blood supply to adipocytes may be reduced, leading to adipocyte hypoxia and necrosis. As a result, macrophage infiltration occurs in adipose tissue which is responsible for the majority of inflammatory cytokine production.¹⁷

Surprisingly, hs-CRP, an indicator of systemic inflammation, decreased in our participants through menopause transition. Since hs-CRP is strongly related to waist circumference in healthy middle aged women³¹ and the fact that the increase in waist circumference over time in our study remained far below the waist circumference thresholds associated with poorer cardiometabolic health² may partly explain the none significant increase in hs-CRP in our sample. Participants in our study showed higher level of physical activity energy expenditure and higher level of dietary fiber intake.⁷ This could also contribute to explain the decrease we observed in hs-CRP during the transition to menopause. Previous research indicates that higher daily physical activity energy expenditure is associated with reduced systemic inflammation (e.g. hs-CRP) in healthy women.³⁹ Studies have shown that high dietary fiber intake could decrease inflammation

especially hs-CRP.⁹⁴ To further investigate the effect of menopause and inflammation in women's health, we investigate the association between inflammatory markers and bone mineral density in premenopausal women transitioning to menopause. Inflammation has been reported to play a role in postmenopausal bone loss.⁶² Progressive estrogen loss during the transition to menopause results in production of cytokines and inflammatory markers.^{62,103} These cytokines can promote bone loss through increasing osteoclast (bone resorption) differentiation, and osteoblast (bone formation) inhibition.^{60,62} Most of scientific evidences on the link between pro-inflammatory markers and bone homeostasis are based on cross sectional studies. Considering that previous studies have shown that plasma levels of inflammatory markers are higher in postmenopausal women compared to premenopausal women⁶, documenting the link between inflammation and bone mineral density in women transitioning to menopause will contribute to determine the potential role of inflammatory markers in bone mineral density loss. The major finding of the present study is that baseline level of hs-CRP is inversely associated with total, lumbar spine and femoral neck bone mineral density in premenopausal women. On the other hand, during the prospective observational follow-up, no association was found between percent changes in inflammatory markers and percent changes in total, lumbar spine and femoral neck bone mineral density. However, significant correlation was found between baseline inflammatory markers and percent changes of total, lumbar spine and femoral neck bone mineral density. Indeed, positive correlation was found between baseline hs-CRP and Hp and percent change of total bone mineral density whereas a negative correlation was observed with baseline IL-6. Also, positive correlation was found between baseline hs-CRP and Hp and percent change of lumbar spine bone mineral density. Finally, there was a positive correlation between baseline hs-CRP and percent change of femoral neck bone mineral density. Meaning the higher the levels

of CRP and Hp at baseline, the lower is the percent decrease in bone mineral density and the higher the level of IL-6 higher is the percent of bone loss. The positive correlation we found was contrary to our expectation that higher inflammation is related to higher bone mineral density loss. This unexpected finding could be partly due to a difference between the actions of inflammatory markers in bone microenvironment and their circulating levels⁷¹ or because of different phenotype of inflammatory markers.¹²⁵ We also reported significant effect of time on bone mineral density (total, lumbar spine and femoral neck), showing an overall decrease throughout the 5 years, while menopausal status per se did not show any significant effect on bone mineral density. However, none of the participants meet the clinical diagnosis criteria of osteoporosis as defined by world health organization criteria. Considering that prevalence of osteoporosis in Canadian women aged ≥ 50 years is 12.1% at the lumbar spine and 7.9% at the femoral neck,¹⁷⁹ the absence of osteoporosis in our sample could be partly explained by high level of physical activity documented in our participants. It is well known that physical activity attenuate bone mineral density loss.¹¹¹

Finally, in premenopausal women hs-CRP, weight and cardiorespiratory fitness determine bone mineral density variations. Baseline level of hs-CRP, Hp, IL-6 and femoral neck bone mineral density along with percent change in physical activity energy expenditure and menopausal status partly explained the individual variation of bone mineral density losses in women transitioning to menopause.

Considering the important role of time spent in the postmenopausal years and years since final menstrual period in the prevalence of the cardiometabolic disorders, a longer follow-up period may be needed for the development of comorbidities normally associated with menopause in healthy women. However, few studies^{23,54,55,148,149} have addressed the influence of years since

final menstrual period on body composition, body fat distribution, inflammatory markers and cardiometabolic risk factors using gold standard methods of measurement. Therefore, we invited the original participants of the MONET cohort to participate in a long-term follow-up study to investigate how body composition, cardiometabolic risk factors, physical activity and the inflammatory markers change after a 10-year follow-up study in healthy normal weight premenopausal women. Our results suggest postmenopausal period is associated with decrease in physical activity energy expenditure, glucose, fat free mass and increase in waist circumference, percent fat mass, total cholesterol and high density lipoprotein. However, only 4% of our participants had metabolic syndrome compared to a prevalence of 19% in Canadian adult population.¹⁸⁰ The changes in body composition could be partly explained by decreased physical activity energy expenditure of our participants due to a shift to a more sedentary life style after menopause. We found a significant negative correlation between years since final menstrual period and percent change in physical activity. In fact, our participants decreased their physical activity energy expenditure by 17% (premenopausal until after 10 years follow up) and 23.2% (since FMP to 10 years) since premenopausal status and final menstrual period, respectively. Another factor that may play a role in menopause related changes in body composition and body fat distribution is the progressive declined estrogen level. Indeed, estrogen deficiency mediates changes in body fat distribution and body composition by increasing fat mass especially visceral fat and decreasing fat free mass¹⁵⁷.

Inflammatory markers including ApoB, ferritin, adiponectin, sCD14 were higher during years after final menstrual period while sTNFR-1 and sTNFR-2 were higher during the final menstruation period and early postmenopausal years. However, the increase of some of the inflammatory markers were still in normal range. The increase in inflammatory markers could be

due to the shift in more fat deposition associated with menopause, which acts as an endocrine organ producing inflammatory markers.⁶ Although we observed an increase of some biomarkers potentially "beneficial" (adiponectin and HDL-C) in healthy premenopausal women going through menopause transition and postmenopausal periods, it is possible that the increase in inflammatory makers and lipids that we observed, may outweigh the beneficial effects and may overcome them in particular over the long term period.

Similar to other health sciences studies, the findings of our study should be interpreted within certain limitations. First, the population studied was composed of healthy women within a narrow range of BMI of $23.2 \pm 2.3 \text{ kg/m}^2$. Therefore, our results are limited to this subgroup of the population and cannot be generalized to the whole population. Still, it is notable to point out that 45% of the women aged between 40 and 59 years in the Canadian population present a BMI between 20 and 29 kg/m^2 .¹⁸¹ Second, the number of participants in the study decreased throughout the study, which reduces the statistical power. Finally, we used the more cost-efficient index (waist circumference) to estimated abdominal fat instead of measuring visceral fat area by computed tomography. Despite these limitations, the present study consists of a well-characterized cohort of women that were followed for 10 years. The longitudinal design allowed us to compare the same women as they went through the menopause transition and postmenopausal years, and thus limit intra-individual variation in factors that could account for the effects on inflammatory markers. Lastly, we used gold-standard methods for the measurement of body composition (dual-energy x-ray absorptiometry), daily physical activity energy expenditure (accelerometer).^{88,182,183}

This thesis enhances our knowledge on menopause issues and its effect on the body weight, body composition, body fat distribution, bone and cardiometabolic health in healthy, non-obese women with relatively higher level of physical activity energy expenditure. As women go through this natural period's of their life, it is of great importance to pay particular attention on having an active lifestyle to maintain a healthy body weight and prevent risk of metabolic syndrome and cardiovascular diseases. Due to the high prevalence of obesity worldwide, which is considered a serious public health problem; the result of this study highlights the importance of developing, implementing and evaluating strategies⁷⁶ to help women maintain a healthy body composition and body fat distribution before, during and after the menopause transition. Tool like weight control decision aids for women¹⁸⁴ to encourage them to have healthier lifestyle practice could be useful, especially during the menopause transition and postmenopausal years to decrease the risk of cardiometabolic diseases.

FUTURE RECOMMENDATIONS

The current research investigated the effect of menopause transition and postmenopausal years on inflammatory markers, body composition, physical activity and bone mineral density among healthy active non obese women. Future research should focus on collecting longitudinal data to compare changes in body composition, diet, physical activity, inflammatory markers and bone mineral density in different phenotype of body weight and physical activity level such as lean active, lean sedentary, obese active, obese sedentary women transitioning to menopause or during postmenopausal years.

CONTRIBUTION

I have been part of the MONET (Montreal Ottawa New Emerging Team) and MONET recall studies on the effect of the menopause transition and postmenopausal years on body composition, inflammatory markers and cardiovascular risk factors. My contributions to these studies especially MONET recall study was numerous and the training that I have been exposed helped me develop and perform the following measurements: resting metabolic rate and thermic effects of food measurement (indirect calorimetry); body composition (DEXA); physical activity energy expenditure (accelerometer) and dietary intake assessment (7 days food journal). I participated in ethics application, participant invitation and recruitment, testing and data collection process, data entry, verification, analysis and interpretation.

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APPENDIX

PUBLISHED PAPERS DURING MY PHD TENURE

Reproducibility of a food menu to measure energy and macronutrient intakes in a laboratory and under real-life conditions.

Jessica McNeil, Marie-Ève Riou, Sahar Razmjou, Sebastien Cadieux, Eric Doucet.

(Br J Nutr. 2012 Oct;108(7):1316-24.)

Author contributions:

Jessica McNeil, Marie-Ève Riou designed the research; Jessica McNeil, Marie-Ève Riou Sahar Razmjou, and Sebastien Cadieux collected the data. Éric Doucet participated in the development of the research project. Jessica McNeil and Marie-Ève Riou performed the analysis and interpretation. All authors were involved in the revision and interpretation of the paper.

Influence of cardiorespiratory fitness and physical activity levels on cardiometabolic risk factors during menopause transition: a MONET study.

Joseph Abdounour, Sahar Razmjou, Eric Doucet, P. Boulay, Martin Brochu, Remi Rabasa-Lhoret, Jean-Marc Lavoie and Denis Prud'homme,

(Prev Med Rep. 2016 Jun 29;4:277-82.)

Author contributions:

Éric Doucet, Martin Brochu, Rémi Rabasa-Lhoret, Jean-Marc Lavoie and Denis Prud'homme participated in the development of the research project (MONET). Joseph Abdounour and Sahar Razmjou participated in the data collection. Joseph Abdounour and Denis Prud'homme performed the analysis and interpretation and completed the writing of the manuscript. All authors were involved in the revision and interpretation of the paper.

Does menarche transition influence or trigger excess weight gain in young adolescent girls? A systematic review

Martin Belliveau, Sahar Razmjou, Dawn Stacey and Denis Prud'homme

(Manuscript in preparation)

Author contributions:

Dawn Stacey and Denis Prud'homme participated in the development of the research project. Sahar Razmjou was the second reviewer of the systematic review. Martin Belliveau and Denis Prud'homme performed the analysis and interpretation and completed the writing of the manuscript. All authors were involved in the revision and interpretation of the paper.