The Diabetes Risk Assessment study:
Elucidating the inflammatory profile of the Metabolically Healthy Obese

by

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ABSTRACT

The Diabetes Risk Assessment study: Elucidating the inflammatory profile of the Metabolically Healthy Obese

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University of Guelph, 2013

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This thesis investigates the complexity of the obesity phenotype by characterizing the inflammatory status of Metabolically Healthy Obese (MHO) individuals. More specifically, this work has examined circulating inflammatory markers in MHO individuals and compared it to Lean Healthy (LH) and Metabolically Abnormal Obese (MAO) subjects. Thirty participants (n=10/group) were recruited as part of the Diabetes Risk Assessment (DRA) study, and classified according to adiposity and metabolic status. Despite a similar level of adiposity compared to MAO individuals, MHO subjects presented a more favourable inflammatory profile. Specifically, MHO individuals had levels of hsCRP and IL-6 comparable to LH subjects and lower than MAO subjects. Also, MHO subjects presented similar levels of high molecular weight adiponectin as the MAO group, but PDGF-ββ levels were intermediate to those of the LH and MAO groups. Overall, the distinct inflammatory profile observed in MHO subjects demonstrates the unique status of these individuals, reinforcing that obesity is a complex and heterogeneous phenotype.
ACKNOWLEDGMENTS

My first thank you goes to my supervisor, Dr David Mutch. His commitment to me went well above the duties expected from an advisor, and knowing he would invest as much time and efforts as I would, made me work the hardest I could, day after day. Thank you to Dr Alison Duncan for accepting to be part of my thesis committee, and to Dr Andrea Buchholz and Dr William Bettger; the three of them kept me up to date with nutrition-related topics, offering new perspectives, and challenging my ideas and conceptions. Thank you to OGS who allowed me to come realize my studies in Ontario and to the Public Health Agency of Canada for funding both of my main projects.

Thank you to my colleagues involved in the Diabetes Risk Assessment (DRA) Study. An enormous thanks to all participants; this research would not have been possible without their involvement in the DRA Study. It was a pleasure to meet all of them, always reminding me that they are the end product of our research efforts. Thanks to the Mutch lab. All of you, in your own way, have made this a memorable journey. We have laughed, we have cried, we have worked hard, we have had fun, but without a doubt, we do achieve great things all together!

Thank you to the very best friends and family; you know who you are. Vaness, who has always believed I could do anything (not everything!). My parents, for supporting me even though I have decided to take the unusual route (en anglais s'il vous plaît!). Marie, for her understanding and wise words (not always what I wanted to hear!). Sarthak, for being this unexpected surprise; I gained more than a degree at Guelph. To all of you: We did it!
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<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AA</td>
<td>Arachidonic acid</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine transaminase</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>AST</td>
<td>Aspartate transaminase</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<td>CV</td>
<td>Coefficient of variability</td>
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<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
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<tr>
<td>DGLA</td>
<td>Dihomo-(\gamma)-linolenic acid</td>
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<tr>
<td>DHA</td>
<td>Docosahexaenoic acid</td>
</tr>
<tr>
<td>DPA</td>
<td>Docosapentaenoic acid</td>
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<tr>
<td>DRA study</td>
<td>Diabetes Risk Assessment study</td>
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<td>DXA</td>
<td>Dual-energy X-ray absorptiometry</td>
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<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>EPA</td>
<td>Eicosapentaenoic acid</td>
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<tr>
<td>FFA</td>
<td>Free fatty acid</td>
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<tr>
<td>GGT</td>
<td>(\gamma)-glutamyltransferase</td>
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<td>HbA1c</td>
<td>Glycosylated haemoglobin</td>
</tr>
<tr>
<td>HDL-c</td>
<td>High-density lipoprotein cholesterol</td>
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<tr>
<td>HMW adiponectin</td>
<td>High molecular weight adiponectin</td>
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<tr>
<td>HOMA%B</td>
<td>Homeostatic model of assessment for (\beta)-cell function</td>
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<tr>
<td>HOMA-IR</td>
<td>Homeostatic model of assessment of insulin resistance</td>
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<td>hsCRP</td>
<td>High sensitive C reactive protein</td>
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<td>IFN-(\gamma)</td>
<td>Interferon-(\gamma)</td>
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<td>IP-10</td>
<td>Interferon-γ inducible protein 10</td>
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<td>LDL-c</td>
<td>Low-density lipoprotein cholesterol</td>
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<td>LH</td>
<td>Lean healthy</td>
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<td>MAO</td>
<td>Metabolically abnormal obese</td>
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<tr>
<td>MCP-1/CCL2</td>
<td>Monocyte chemotactic protein / Chemokine (C-C motif) ligand 2</td>
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<tr>
<td>MHO</td>
<td>Metabolically healthy obese</td>
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<tr>
<td>MONW</td>
<td>Metabolically obese normal weight</td>
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<tr>
<td>MUFA</td>
<td>Monounsaturated fatty acid</td>
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<tr>
<td>n-3 PUFA</td>
<td>Omega 3 polyunsaturated fatty acid</td>
</tr>
<tr>
<td>n-6 PUFA</td>
<td>Omega 6 polyunsaturated fatty acid</td>
</tr>
<tr>
<td>NCEP-ATP III</td>
<td>National cholesterol education program-Adult treatment panel III</td>
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<tr>
<td>NFκB</td>
<td>Nuclear factor κ-light-chain-enhancer of activated B cells</td>
</tr>
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<td>OGGT</td>
<td>Oral glucose tolerance test</td>
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<tr>
<td>PDGF-ββ</td>
<td>Platelet derived growth factor-ββ</td>
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<tr>
<td>PUFA</td>
<td>Polyunsaturated fatty acid</td>
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<tr>
<td>RANTES/CCL5</td>
<td>Regulated upon activation normal T-cell expressed and secreted / Chemokine (C-C motif) ligand 5</td>
</tr>
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<td>SEM</td>
<td>Standard error of the mean</td>
</tr>
<tr>
<td>SFA</td>
<td>Saturated fatty acid</td>
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<tr>
<td>T2D</td>
<td>Type 2 diabetes</td>
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<tr>
<td>TG</td>
<td>Triglyceride</td>
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<tr>
<td>TLR-2/4</td>
<td>Toll-like receptor 2 and 4</td>
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<tr>
<td>TNF-α</td>
<td>Tumour-necrosis factor</td>
</tr>
<tr>
<td>TNH study</td>
<td>Toronto Nutrigenomic Health study</td>
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<td>Total-c</td>
<td>Total-cholesterol</td>
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Chapter I

Obesity: More Than Meets the Eye (and Weight Scale)
Chapter I Summary

Weight on the scale is not the sole determinant of health status and risk of downstream complications. Not all obese individuals display metabolic abnormalities, and not all lean individuals present a healthy metabolic profile. This paradox highlights the need to better understand the heterogeneous obesity phenotype and the causes and consequences related to excess body weight. Accordingly, distinct subgroups of individuals are now commonly discussed in the literature, and referred to as i) Metabolically Obese Normal Weight (MONW), ii) Metabolically Healthy Obese (MHO), and iii) Metabolically Abnormal Obese (MAO).

The goal of this chapter is to summarize the current state of knowledge regarding the heterogeneous obesity phenotype and highlight what is known about the MONW, MHO and MAO subgroups. Specifically, the present chapter provides an up-to-date overview of the literature demonstrating evidence that these groups differ in a number of critically important parameters that are relevant for obesity, including body composition, energy metabolism, inflammation, and adipose tissue morphology.
1. Introduction

At a time when obesity has reached epidemic proportions, research now shows that it is not simply a person’s weight on the scale that determines their health status and risk for downstream complications. Indeed, not all obese individuals display metabolic abnormalities and not all lean individuals have a healthy metabolic profile [1]. This paradox reinforces the notion that excess body weight is not the sole determinant of complications like type 2 diabetes (T2D), hypertension, and cardiovascular diseases (CVD) [1].

As research aims to better understand the heterogeneous obesity phenotype and the causes and consequences related to excess body weight, distinct subgroups of individuals have been classified. These subgroups, which are now more commonly discussed in the literature, are referred to as i) Metabolically Obese Normal Weight (MONW), ii) Metabolically Healthy Obese (MHO), and iii) Metabolically Abnormal Obese (MAO) (see Box 1 for alternate nomenclature). These subgroups are not classified using simply body weight or body mass index (BMI), but instead are classified using numerous measurements that reflect their overall metabolic health. However, most of the research conducted to date on human obesity classifies subjects into lean and obese groups based on recognized BMI cut-offs, which often fails to accurately reflect the metabolic status of an individual [1]. This can have important and widespread ramifications on both Metabolically Obese Normal Weight individuals (MONW)
We recognize that a similar subgroup has been identified in the literature; the normal weight obese (NWO) phenotype [2, 3, 6-10, 44] who seems to be characterized by a high percentage of body fat despite a normal body weight. NWO subjects display a cluster of metabolic abnormalities without being classified as having the metabolic syndrome. For the purpose of this work, no distinction will be made between the MONW and the NWO groups.

Metabolically Healthy Obese individuals (MHO)
This subgroup is also referred as the Metabolically normal obesity group [20, 75, 94], the Metabolically benign obesity group [53, 71, 74, 77, 97], the Insulin-sensitive obesity group [57, 63], or the Metabolically healthy but obese group [15].

Box 1: Alternate Nomenclature.
basic and clinical research related to obesity. Therefore it is critically important that an individual’s metabolic status be considered in order to correctly assess their risk for obesity-related complications and ensure optimal health management. While body weight and BMI are certainly useful indicators, the inclusion of measurements to evaluate an individual’s metabolic status appears necessary as we seek to better understand the genetic and molecular basis for obesity.

The goal of this chapter is to summarize the current state of knowledge regarding the heterogeneous obesity phenotype and highlight what is known about the MONW, MHO and MAO subgroups. Specifically, this chapter provides an up-to-date overview of the literature providing evidence that these groups differ in a number of critically important parameters that are relevant for obesity, including body composition, energy metabolism, inflammation, and adipose tissue morphology.

2. Identification of the MONW and MHO groups

Perhaps the most significant obstacle that exists presently concerns the methods used to classify individuals into the various subgroups. Indeed, there is currently no consensus with regards to which method should be used to classify subjects, making it challenging to compare literature in the area. Overall, the various methods used to classify individuals into MONW, MHO, and MAO groups use a combination of a person’s adiposity status (e.g., BMI, percentage body fat and/or waist circumference) coupled with their metabolic status (e.g., estimates of insulin sensitivity, blood lipid levels, and/or markers of inflammation). However, the measurements used to classify individuals vary widely from one report to another.

The lean healthy (LH) group is considered the “optimal” group with regards to adiposity and metabolic status, and is often defined as the control or reference group in research protocols.
LH individuals have a BMI between 18.5 and 25 kg/m² and have a low percentage body fat. In contrast, individuals with a BMI ≥ 30 kg/m² are considered obese and usually have a high percentage body fat. These obese individuals typically show numerous metabolic abnormalities that put them at higher risk of developing T2D and CVD. This group is widely considered to be the MAO subgroup. While the LH and MAO subgroups can be considered to lie at the extremes on the continuum of body weight, it is what lies between these two subgroups that has proved difficult to robustly define.

**Metabolically Obese Normal Weight:** Individuals classified as MONW show metabolic abnormalities despite having a BMI that is similar to LH subjects. Table 1 provides an overview of different methods used to identify MONW subjects. Most studies agree that individuals will be classified as MONW if they have a BMI less than either 25 kg/m² [2-10] or 27 kg/m² [11, 12]. MONW also have a high percentage body fat, determined using either a general cut-off point of > 30% [2, 7, 9, 10] or sex-specific tertiles (e.g., highest tertile for men and women, corresponding to > 23% and > 33% respectively [3, 6]). As outlined in Table 1, various measures have been used to define the metabolic status for MONW. In 1998, Ruderman et al. suggested using a combination of 18 measurements (e.g., predisposing factors, weight history, metabolic factors, etc.) each associated with a number of points proportionate to the health risks; where a final score > 7 resulted in an individual being considered as MONW [11]. While thorough, this classification method remains fastidious because markers such as weight history and birth weight are difficult to obtain from study participants. Therefore the metabolic status of MONW is typically defined using cardiometabolic measurements in accordance with the definition criteria for metabolic syndrome used by the National Cholesterol Education Program-
Adult Treatment Panel III (NCEP-ATP III). Specifically, a person is considered MONW if they meet 3 or more of the following criteria: waist circumference > 102cm for males and > 88 cm for females; blood pressure > 130/85 mmHg not treated; triglycerides (TG) ≥ 1.7 mmol/L; high-density lipoprotein cholesterol (HDL-c) ≤ 1.03 and 1.3 mmol/L for males and females respectively; and fasting glycemia ≥ 6.2 mmol/L not treated, as described by St-Onge et al. [13]. Others have classified MONW using a hyperinsulinemic-euglycemic clamp to measure insulin sensitivity. Using this precise technique, researchers have either used a cut-off value lower than 8 mg/min•kg lean body mass for impaired insulin sensitivity [14] or divided their population into quartiles based on glucose disposal rates and considered those in the lowest quartile to be MONW (corresponding to a value < 10.2 mg/min•kg lean body mass) [5]. While clamps are considered the gold-standard by which to assess insulin sensitivity, they are onerous to perform and not commonly used in large population-based studies [15]. Instead, many researchers measure fasted insulin and glucose levels, and subsequently use these values to mathematically estimate an individual’s insulin sensitivity using the Homeostatic model of assessment of insulin resistance (HOMA-IR). With this approach, MONW have been classified as individuals above a specific HOMA-IR cut-off of 1.69 [16] or in the highest quartile of the study cohort [17, 18].

Metabolically Healthy Obese: In contrast with MONW individuals, people classified as MHO have a BMI comparable to MAO individuals but show no signs of metabolic abnormalities [19]. Table 2 provides an overview of the various methods used to classify MHO subjects. MHO individuals have a BMI > 30 kg/m² [5, 20-25]. While most researchers use BMI to identify MHO subjects, some groups have questioned whether this measure accurately reflects the percentage body fat [26, 27]. For example, Brochu et al. did not consider BMI when studying
postmenopausal women because this index was deemed inadequate in older people, and in particular postmenopausal women, due to increased fat mass and decreased lean mass associated with aging [27]. Instead, these authors preferred to measure percentage body fat, which is a more accurate measure of adiposity. However, as pointed out by Velho et al., the lack of an internationally accepted cut-off for percentage body fat to define obesity currently limits the use of this measurement for classification purposes [28]. Thus while BMI may not be the most accurate marker for body fat, it remains a more accessible and recognized measurement in clinical care units than percentage body fat.

As outlined in Table 2, the most common measure of metabolic status used to identify MHO individuals involves assessing insulin sensitivity. Some research groups have used the hyperinsulinemic-euglycemic clamp as a measure of insulin sensitivity, where cohorts are separated into tertiles [29] or quartiles [5, 30, 31], and the upper tertile/quartile corresponds to MHO individuals. Others have preferred the use of a set cut-off point, where high insulin sensitivity is generally defined by a value greater than 8 mg/min•kg lean body mass [27, 32, 33]. Still others have used an oral glucose tolerance test (OGTT), where MHO is defined as individuals in the upper quartile of insulin sensitivity [34-36]), or the HOMA-IR index, where MHO is defined as individuals in the lowest tertile [17, 37].

As demonstrated by the previous discussion, there is currently no consensus regarding the criteria used to identify MHO individuals. Recently, Messier et al. compared different classification methods in a population of 113 obese sedentary postmenopausal women; methods that reflect the ones commonly seen in the literature [38]. Specifically, the authors compared the following five methods: i) clamp technique, where MHO individuals corresponded to those in the upper quartile of glucose disposal rate, ii) OGTT and Matsuda index, where MHO
individuals corresponded to those in the upper quartile for the Matsuda index, iii) HOMA-IR index, where MHO individuals corresponded to those in the lowest quartile of insulin resistance, iv) cardiometabolic parameters, where MHO individuals had ≥ 4 out of 5 criteria in the normal range according to NCEP-ATP III criteria, and v) factors focused on lipidemia and inflammation, where MHO subjects had ≥ 4 out of 5 criteria (HOMA-IR ≤ 2.7, TG ≤ 1.7 mmol/L, HDL-c ≥ 1.3 mmol/L, low-density lipoprotein cholesterol (LDL-c) ≤ 2.6 mmol/L and high sensitive C reactive protein (hsCRP) ≤ 3.0 mg/L). When comparing these different classification methods, 42% of their population was classified as MHO by at least one method, while 27%, 21%, 10%, 0% were classified as MHO by two, three, four, or five methods, respectively. This study showed that variations in body composition and metabolic risk factors are highly dependent on the methods used to identify MHO individuals, thus reinforcing the need to have an expert consensus to standardize definition criteria.

In summary, the lack of established criteria complicates the classification of MONW and MHO individuals and renders comparisons between studies difficult. Together, this highlights the absolute necessity to establish criteria that include both measures of adiposity status as well as measures of metabolic status. However, it is key that a consensus is reached that finds a suitable middle ground to ensure scientific robustness and clinical practicality. To define adiposity status, BMI or waist circumference measurements should still be used, but sex- and age-specific thresholds must be determined [39, 40]. To characterise metabolic status, a set of criteria should be used that includes parameters related to lipid and glucose homeostasis.
Table 1: Overview of the various definition criteria used to identify MONW individuals. This table summarizes the various criteria used by authors to identify the MONW subgroup. Each definition is a combination of markers of the adiposity status and the metabolic status; the minimum number of criteria required to be classified as a MONW individual is indicated in the bottom row ('All' indicates that all the listed factors need to be met to be classified as MONW). As a reference point, the definition suggested by St-Onge et al. is the one used to define the metabolic syndrome (as established by the NCEP-ATP III) where 3 or more criteria need to be met.

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<tbody>
<tr>
<td><strong>BMI [kg/m²]</strong></td>
<td>Score</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>*Asian female population</td>
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</tr>
<tr>
<td>Waist circumference [cm]</td>
<td>1.2</td>
<td>102 (M), &gt; 98 (F)</td>
<td>&gt; 96-91, &gt; 91 (M) 30-76, &gt; 76 (F)</td>
<td>&gt; 90 (F)</td>
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<td>≥ 130/85 or treated</td>
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<td>TG [mmol/L]</td>
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<td>≥ 1.7</td>
<td>≥ 1.7</td>
<td>≥ 1.7</td>
<td>≥ 1.7</td>
<td>≥ 1.7</td>
<td>≥ 1.7</td>
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<tr>
<td>HDL-c [mmol/L]</td>
<td></td>
<td></td>
<td>&lt; 0.9 and TG &gt; 1.7</td>
<td>≥ 1.03 (M), &gt; 1.3 (F)</td>
<td>≤ 1.03 (M), &lt; 1.3 (F)</td>
<td>≤ 1.03 (M), &lt; 1.3 (F)</td>
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<td>≤ 1.03 (M), &lt; 1.3 (F)</td>
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<td>Hyperglycemia</td>
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<td></td>
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<td>Clamp, Cut-off &lt; 8 mg/ min x kg lean body mass</td>
<td>Clamp, Lowest Quartile ≤ 10.2mg/min x kg lean body mass</td>
<td>Clamp, Lowest Quartile ≤ 10.2mg/min x kg lean body mass</td>
<td>Clamp, Lowest Quartile ≤ 10.2mg/min x kg lean body mass</td>
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<td>hsCRP [mg/L]</td>
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<td>Visceral adipose tissue (cm²)</td>
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<td>Weight history</td>
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<tr>
<td>Minimal number of criteria to be identified as MONW</td>
<td>Score ≥ 7</td>
<td>All</td>
<td>All</td>
<td>All</td>
<td>≥ 3 + BMI</td>
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<td>≥ 2 + BMI</td>
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Table 2: Overview of the various definition criteria used to identify MHO individuals. This table summarizes the various criteria used by authors to identify the MHO subgroup. Each definition is a combination of markers of the adiposity status and the metabolic status; the minimal number of criteria required to be classified as an MHO individual is indicated in the bottom row (‘All’ indicates that all the listed factors need to be met to be determined as MHO). As a reference point, the definition suggested by Meigs et al. is the one used to define the metabolic syndrome (as established by the NCEP-ATP III) where 3 or more criteria need to be met.

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<td>Adiposity status</td>
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<td>BMI [kg/m²]</td>
<td>≥ 30</td>
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<td>≥ 30</td>
<td>*Asian population ≥ 25</td>
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<td>Waist circumference [cm]</td>
<td>&lt; 102 (M), &lt; 88 (F)</td>
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<td>Body fat [%]</td>
<td>≥ 35 (F)</td>
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<td>Metabolic status</td>
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<td>Blood pressure [mmHg]</td>
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<td>&lt; 130 (F), not treated</td>
<td>&lt; 130 (F), not treated</td>
<td>≤ 130 (M), not treated</td>
<td>&gt; 140/90, not treated</td>
<td>≤ 130 (M), not treated</td>
<td>&gt; 140/90, not treated</td>
<td>≤ 130 (M), not treated</td>
<td>&gt; 140/90, not treated</td>
<td>≤ 130 (M), not treated</td>
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<td>TG [mmol/L]</td>
<td>&lt; 1.7</td>
<td>TG/HDL-c &lt; 3</td>
<td>&lt; 1.7</td>
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<td>Total cholesterol [mmol/L]</td>
<td>&lt; 5.2</td>
<td>Total&lt;;HDL-c &lt; 4.4</td>
<td>&lt; 5.2</td>
<td>&lt; 5.2</td>
<td>&lt; 5.2</td>
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<td>LDL-c [mmol/L]</td>
<td>&lt; 2.6, not treated</td>
<td>&lt; 2.6</td>
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<td>&lt; 2.6</td>
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<td>HDL-c [mmol/L]</td>
<td>≥ 1.3, not treated</td>
<td>≥ 1.0 (M), ≥ 1.3 (F), not treated</td>
<td>≥ 1.0</td>
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<td>≥ 1.3</td>
<td>≥ 1.0 (M), ≥ 1.3 (F), not treated</td>
<td>≥ 1.0</td>
<td>≥ 1.3</td>
<td>≥ 1.0 (M), ≥ 1.3 (F), not treated</td>
<td>≥ 1.0</td>
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<td>Hyperglycaemia</td>
<td></td>
<td>Clamp: Cut-off ≥ 8 mg/l x kg lean body mass</td>
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<td>Clamp: Top quartile ≥ 12.6 mg/l x kg lean body mass</td>
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<td>Clamp: Top quartile ≥ 12.6 mg/l x kg lean body mass</td>
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<td>Clamp: Top quartile ≥ 12.6 mg/l x kg lean body mass</td>
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<td>Clamp: Top quartile ≥ 12.6 mg/l x kg lean body mass</td>
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<td>Fasting glucose [mmol/L]</td>
<td>&lt; 5.6</td>
<td>&lt; 6.2 and not treated</td>
<td>&lt; 5.6</td>
<td>&lt; 5.6</td>
<td>&lt; 5.6</td>
<td>&lt; 5.6</td>
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<td>Fasting insulin [pmol/L]</td>
<td>&lt; 90</td>
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<td>HOMA-IR</td>
<td>Clamp, top quartile ≤ 0.15</td>
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<td>hsCRP [mg/L]</td>
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<tr>
<td>Family history and Predisposing factors</td>
<td>No history of cardiovascular, respiratory or metabolic disease</td>
<td>No history of cardiovascular, respiratory or metabolic disease</td>
<td>No history of cardiovascular, respiratory or metabolic disease</td>
<td>No history of cardiovascular, respiratory or metabolic disease</td>
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<tr>
<th>Minimal number of criteria to be identified as MHO</th>
<th>All</th>
<th>≥ 4 + BMI</th>
<th>All</th>
<th>≥ 3 + BMI</th>
<th>≥ 4 + BMI</th>
<th>≥ 4 + BMI</th>
<th>≥ 5 + BMI</th>
<th>All</th>
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*Note: BMI = Body Mass Index, MHO = Metabolically Healthy Obese, TG = Triglycerides, HDL-c = High-Density Lipoprotein cholesterol, LDL-c = Low-Density Lipoprotein cholesterol, HOMA-IR = Homeostatic Model Assessment of Insulin Resistance, hsCRP = High-Sensitivity C-Reactive Protein.*
3. Prevalence of the MONW and MHO phenotypes

Due to the lack of defined criteria to identify each phenotype, it is understandable that the prevalence of MONW and MHO has been difficult to estimate. However, the estimates of prevalence of these two groups that have been reported in different cohort and populations to date supports the need to better characterize these MONW and MHO individuals as they may represent significant subgroups of the actual obesity pandemic.

The prevalence of MONW has been estimated to range from ~10% based on studies in Caucasian American and Canadian cohorts [1, 17] to as high as 37% based on a study in a Venezuelan population [41]. As for the prevalence of MHO, it has been estimated to range between 4% to 29% depending on the population studied, with the lowest estimate identified in a Dutch population [42] and the highest in a Korean population [43]. These estimates underscore possible ethnic differences, and could be linked to genetic makeup, lifestyle, as well as environmental factors. However, it is noteworthy that these studies have not used the same definition criteria, making it difficult to directly compare the prevalence amongst different cohorts or populations. Indeed, a relaxed definition of the metabolic status (e.g., meeting 3 out of 5 cardiometabolic factors) will generate a higher estimate of prevalence compared to a more strict definition (e.g., cut-off for HOMA-IR).

Interestingly, recent findings suggest that the prevalence of the MONW phenotype may differ for men and women. For example, a study by Marques-Vidal et al. reported that the prevalence of MONW among middle-aged men was estimated to be ~1% irrespective of the criteria used, while the prevalence in women ranged from 1% using a sex-specific threshold to 28% using a age- and sex-independent cut-off point (MONW defined as 1. BMI < 25 kg/m² sex-specific percentage body fat > 26% for men and > 43% women, or 2. BMI < 25 kg/m² an age-
and sex-independent percentage body fat > 30% for men and women) [44]. Moreover, Wildman et al. estimated the prevalence of MONW to be 17% in a population of older women aged 59 to 70 years [45], which is slightly higher compared to the prevalence of 13% in the general population [1]. It is hypothesized that the higher estimates of prevalence for MONW in women stems from their predisposition for increased body fat [6]. Further, the higher estimates noted for post-menopausal women suggests a possible role for sex hormones in the MHO phenotype [7].

The prevalence of MHO has also been reported to be higher among women: 65% of women vs 46% of men being identified as MHO within the general Canadian population [46]. Overall, these results highlight the need to define sex-specific criteria and cut-offs in order to more robustly estimate the prevalence of these different obesity phenotypes within the population [3, 44].

Unsurprisingly, how adiposity status and metabolic status are defined can have a significant influence on estimates of phenotype prevalence. Shea et al. studied the prevalence of the MHO phenotype in a large Canadian cohort using either BMI or percentage body fat as classification criteria for adiposity status [46]. The authors showed that there was little agreement in the estimates when comparing BMI and percentage body fat, and that the prevalence of MHO was higher when they used percentage body fat. This was also recently confirmed by Shah et al., who showed that 39% of the middle-aged men and women in their cohort who were considered lean using BMI were in fact classified as obese when using percentage body fat as measured by dual-energy X-ray absorptiometry (DXA) [39]. Taken together, these reports suggest that using a more precise method to determine excess fat in comparison to BMI will enable the research community to reach a consensus regarding the prevalence of the MONW and MHO phenotypes.
Overall, despite the lack of clear definition and optimal methods to assess the prevalence of the MONW and MHO groups, current evidence demonstrates their existence as distinct groups to the LH and MAO phenotypes. As such, this reinforces the need to study these individuals in order to deepen our understanding of the obesity phenotype.

4. Body Composition and Fat Distribution

Body fat distribution is believed to be at least, if not more, important than total body fat when it comes to understanding the basis for the metabolic disturbances seen with obesity [47]. Accordingly, studying body composition and fat distribution could help to understand the different groups of obese individuals and their risks for downstream complications.

As depicted in Figure 1, MONW subjects have a BMI within the normal range; however, BMI is considered a poor marker to identify MONW individuals as it fails to capture the metabolic risk associated with an increased percentage body fat [14]. As such, MONW individuals can remain undetected when using basic measurements such as body weight, BMI, and skinfold thickness measurements [12]. Conus et al. found no differences between a group of MONW individuals and LH subjects of equivalent body weight for nine measured skinfold tests, as well as waist, hip, and thigh circumference measurements [16]. However, it has been shown that MONW women have a percentage body fat as well as a fat distribution profile similar to obese individuals [9]. These findings suggest that routine measurements in medical care units may be missing the increased metabolic risk of MONW individuals, and should be complemented with more robust analyses of body composition.
It is plausible that visceral fat could be the primary criterion with which to distinguish MONW individuals from LH individuals [48]. A previous study reported that MONW individuals had increased visceral fat depots compared to LH individuals [11]. While this hypothesis requires further confirmation, increased visceral fat is a plausible explanation to justify the increased metabolic risk seen in MONW individuals. Indeed, the accumulation of visceral fat, rather than obesity per se, is associated with cardiometabolic risk factors such as hypertension and insulin resistance, as observed in the MAO phenotype [49]. Moreover, visceral fat secretes inflammatory cytokines (e.g., TNF-α and IL-6) and free fatty acids (FFA) into portal and peripheral circulation, promoting hepatic steatosis and hyperinsulinemia [1, 11]. The

**Figure 1: Continuum of adiposity status and metabolic status.** The Metabolically Obese Normal Weight (MONW) group presents an unhealthy metabolic status despite showing a healthy weight. On the other hand, the Metabolically Healthy Obese (MHO) group presents a healthy metabolic status despite showing an obese state. The Metabolically Abnormal Obese (MAO) group presents both an unhealthy weight status and metabolic status when compared to lean healthy (LH) individuals. MONW, MHO and MAO individuals show early signs of complications and/or are at higher risks of developing both physiological and metabolic complications usually associated with obesity.
mobilization of FFA from visceral fat also results in the ectopic storage of fat in tissues such as muscle and the pancreas, thus compromising the normal function of these tissues [50].

Subjects considered MHO have higher values of BMI, fat mass, and waist circumference compared to LH individuals [5]. When compared to MAO individuals, MHO individuals do not differ significantly for BMI [5, 23, 31, 34], but have lower waist circumference [5, 23]. For example, Cherquaoui et al. reported that MHO individuals had a lower waist circumference and waist-to-hip ratio compared to MAO individuals, suggesting that these measurements could be adequate anthropometric measurements to distinguish the MHO phenotype from the MAO group [23]. In another study, Messier et al. showed that despite MHO and MAO postmenopausal women having similar BMI, waist circumference and fat mass, individuals characterized as MHO had significantly less visceral adipose tissue [34]; suggesting that MHO individuals may store fat differently compared to MAO individuals.

People classify as MHO have an increased body weight but an overall “healthier” cardiometabolic status. It has been proposed that the location of fat storage may explain this seemingly paradoxical situation, as visceral obesity is now widely recognized as a major risk factor contributing to metabolic disease [47]. Some have suggested that MHO subjects have lower amounts of visceral adipose tissue, thus explaining their preserved metabolic functions. When compared to MAO individuals with a similar percentage body fat, MHO were found to have ~50% less visceral adipose tissue [19, 27, 38]. It has been hypothesized that MHO may preferentially store excess fat in subcutaneous adipose tissue depots and that this could provide a partial explanation for their preserved insulin sensitivity [38, 51]. However, this is not without controversy given that multiple studies have found no difference in subcutaneous adipose tissue depots between MHO and MAO individuals [27, 52-54]. Whether or not the fat distribution
could explain their healthy profile, the site of fat storage remains to be investigated as some studies have shown that MHO individuals also present less ectopic fat accumulation in the liver and skeletal muscles [19, 53, 55]. Taken together, this reinforces the importance of elucidating if the fat distribution profile in MHO individuals confers protection from complications usually associated with obesity.

As described, rather than using BMI as an indirect measure of body fat, the predominance of various fat depots and body fat distribution may be better predictors regarding an individual’s risk of developing obesity-related complications. Accordingly, body fat distribution should be carefully assessed in order to better understand the MONW and MHO phenotypes.

5. Glycemia and Insulinemia

It is widely accepted that obesity is associated with impaired glycemia due to decreased insulin secretion and/or insulin sensitivity [56]; however, this situation more accurately reflects the comparison between LH and MAO individuals. Evidence is accumulating showing that MONW individuals have a body weight comparable to LH individuals, but have lower insulin sensitivity, while MHO individuals have a body weight comparable to MAO individuals, but have higher insulin sensitivity. This demonstrates that the glycemic status of MONW and MHO individuals do not align with simple measures of body weight.

MONW individuals have been characterized as hyperinsulinemic, which could be an early indicator of a pre-diabetic state [11, 56]. In a cohort of twelve MONW women (defined by a HOMA-IR value $\geq 1.69$) between the ages of 18 to 35 years, it was shown that 17% of the variance in the HOMA-IR index could be attributed to the percentage body fat [16]. Although little evidence exists, MONW individuals can experience insulin sensitivity impairment, and it seems to be partly due to their excess percentage body fat. Accordingly, more studies are
required to characterize the MONW glucose metabolism and how, despite their relatively normal BMI, they develop insulin resistance.

In contrast, MHO individuals have been found to have an insulin sensitivity that is comparable to that of LH individuals, as measured by an OGTT where MHO was defined by insulin sensitivity values in the upper quartile [53]. When compared to MAO individuals, MHO subjects were shown to have higher insulin sensitivity, lower values of glycosylated haemoglobin (HbA1c), and lower fasting glucose and insulin values [19, 57]. One hypothesis to explain why MHO individuals have preserved insulin sensitivity despite their increased body weight is related to pancreatic β-cell function. After adjusting for BMI, MHO individuals were found to have a β-cell function comparable to LH patients [5]. This suggests that obesity per se does not necessarily cause β-cell dysfunction. Indeed, other factors such as adipocyte secretion of pro-inflammatory molecules or secretion of FFA could impair β-cells, and eventually lead to lipotoxicity and/or reduced insulin secretion [5].

Although it is recognized that excess fat is associated with insulin resistance [58], the glycemic status of the MONW and MHO groups does not support it, and reinforces the urgent need to define measures of body weight and fat distribution that will better reflect the metabolic status and associated risks of these individuals.

6. Lipidemia

Excess body fat is widely associated with circulating lipid perturbations that increase an obese individual's risk for related complications such as T2D and CVD [59].

Despite their normal BMI, MONW individuals were found to have elevated TG, LDL-c, and reduced HDL-c levels in comparison to LH individuals [1, 3, 6, 12, 16]. Based on this lipidomic profile, MONW subjects tend to resemble MAO individuals more than LH individuals.
Along these lines, Succurro et al. reported no differences for total-cholesterol (Total-c), LDL-c or FFA between MONW and MAO subjects [5]. Accordingly, the atherogenic lipid profile of MONW individuals also increased their prevalence for diagnosed dyslipidemia [18].

Interestingly, MHO individuals appear to have a lipidomic profile that falls between LH and MAO individuals [60, 61]. When compared to MAO individuals with similar body fat, MHO individuals have been shown to have lower TG and higher HDL-c levels [19, 27, 31, 46, 57, 62]. Another study also reported that MHO individuals have reduced Total-c and LDL-c compared to MAO individuals [54]; however, this has not been reported in all studies [31]. While these conflicting studies may be explained by differences in study population (i.e., older post-menopausal women with a mean BMI of 33 kg/m² vs middle-aged women with a mean BMI of 27 kg/m²), these results suggest that more specific markers of lipid metabolism should be used in order to more accurately assess an individual’s risk for metabolic complications. One avenue of interest would be to conduct global lipidomic analyses on blood, which may help identify associations between specific lipid intermediates and insulin sensitivity amongst the different obesity phenotypes [63]. However, these analyses are highly dependent on the criteria used to define the subgroups, especially because circulating lipids tend to be widely used as part of the criteria to identify MONW and MHO individuals (Tables 1 and 2).

As excess body fat is associated with perturbations in circulating lipids, more research is needed to understand the MONW and MHO groups, and to determine identification criteria that will reflect their metabolic status and associated risks.

7. Adipose Tissue Characteristics

Adipose tissue is now a recognized endocrine organ that plays a central role in the development of the obesity-related complications [64]. Therefore, its characterisation in the
MONW and MHO phenotype would permit a better understanding of the relationship between adipose tissue, low-grade inflammation and insulin resistance.

Srdic et al. analysed abdominal adipose tissue morphology in the MONW group [60]. When compared to LH subjects, the authors noted adipocyte hypertrophy in visceral adipose tissue in the MONW group. As adipocytes undergo hypertrophy and, eventually reach their maximal size, they become susceptible to hypoxia and, subsequently, to cell death [60]. In fact, complications associated with obesity such as insulin resistance and dyslipidemia have been previously linked to adipose tissue dysfunction [51]. Indeed, hypertrophic obesity (i.e., increased volume of existing adipocytes), as opposed to hyperplastic obesity (i.e., increased number of adipocytes), is more likely to lead to insulin resistance and T2D due to hypoxia, dysregulation in adipokine secretion, and inflammation [57, 62] (Figure 2). Despite being obese, MHO individuals seem to be protected from metabolic abnormalities and that could be due to their preserved adipose tissue function [51]. Dysfunctional adipose tissue is typically characterized by increased macrophage infiltration; however, MHO individuals have reduced macrophage infiltration in visceral adipose tissue compared to MAO individuals [51]. At the cellular level, differences between MHO and MAO individuals have also been observed in adipocytes. McLaughlin and colleagues showed that MHO individuals had a two to three fold increase in the expression of genes related to adipocyte differentiation compared to MAO individuals [62]; suggesting that MHO have adequate hyperplasia possibly preserving their adipose tissue functions. This result is consistent with the hypothesis that MHO would present a “healthier” adipose tissue with suitable adipokine secretion and preserved insulin sensitivity [60]. Moreover, it has been hypothesized by Wildman and colleagues that MHO individuals would either not possess the same adipose tissue endocrine secretory properties as MAO individuals or they could
be less responsive to endocrine secretions of excess adipose tissue; possibly explaining their healthy status despite their obesity [61].

Taken together, future efforts to better understand the MONW and MHO phenotypes should focus on the characterization of adipose tissue as an endocrine organ and generate more knowledge about different fat depots, adipocyte differentiation, and the secretion of adipokines [57, 62].

![Figure 2: Overview of the interaction between adipose tissue remodelling, low-grade inflammation and insulin resistance.](image)

**Figure 2: Overview of the interaction between adipose tissue remodelling, low-grade inflammation and insulin resistance.** White adipose tissue (comprised of visceral and subcutaneous depots) undergoes hypertrophy (increased size of the actual adipocytes) and hyperplasia (increased number of new adipocytes). Hypertrophy ultimately leads to low-grade inflammation and insulin resistance.

### 8. Circulating Adipokine Profile and Inflammatory Profile

It is now recognized that obesity is characterized as a low-grade inflammatory state mainly due to adipose tissue dysfunction [65]. Adipose tissue secretes numerous adipokines that affect insulin sensitivity, inflammation and energy metabolism [58]. In the context of excess caloric intake where the adipose tissue undergoes remodelling, altered adipokine secretion could be a link between dysfunctional adipose tissue and obesity-related complications [64]. Therefore,
measuring the circulating concentration of adipokines as a reflection of the “health status” of the adipose tissue could deepen our understanding of the MONW and MHO phenotypes.

Di Renzo et al. reported that MONW subjects have circulating levels of adipokines (IL-1α, IL-1β, IL-6, IL-10, IL-15, and TNF-α) comparable to those observed in MAO individuals; thus reinforcing the notion that inflammation, rather than body weight, may be the dominant precursor to the development of metabolic complications [9]. The increased inflammatory profile of MONW individuals was also observed by De Lorenzo et al., who reported an increase in a panel of circulating cytokines in a group of young MONW women in comparison to LH women [2]. Importantly, De Lorenzo and colleagues did not observe differences in hsCRP between MONW and LH women, suggesting that using a marker for whole-body inflammation may be insufficient to capture the altered inflammatory state associated with the MONW phenotype [2].

Low-grade inflammation has also been investigated in MHO individuals; however, there is a lack of consensus within the literature. Some studies have shown that MHO individuals have higher hsCRP levels compared to LH individuals [46] and MONW subjects [66], but lower than MAO individuals [31, 52, 57]. In contrast, Messier et al. found no significant differences in hsCRP between MHO and MAO individuals in their cohort [34]. These conflicting results are intriguing given that the study populations used by Dai et al. [31] and Karelis et al. [52] were similar to the one studied by Messier and colleagues; both studied a cohort of post-menopausal women where MHO were defined using BMI and the hyperinsulinemic-euglycemic clamp. In yet another study, Shin et al. reported that MHO individuals had reduced plasma CRP levels, as well as IL-6, compared to MAO individuals [54]. A possible explanation for this result is that CRP synthesis is highly regulated by IL-6, which is preferentially secreted by visceral adipose tissue. Therefore, one can speculate that the reduced visceral adipose tissue depot in MHO
individuals seen by Shin et al. could be the underlying basis for the lower IL-6 and CRP levels [54].

Recent studies have also examined key adipokines (adiponectin, leptin and resistin) amongst MHO and MAO individuals with similar BMI. Several studies have reported that MHO individuals have elevated levels of circulating total adiponectin [57, 67] or high molecular weight adiponectin (HMW adiponectin) [29], similar to LH individuals. Considering the insulin sensitizing functions of adiponectin, studying it in the different obesity phenotypes is of interest as it could provide details on excess fat and insulin resistance.

While existing data suggest that differences in inflammatory status may distinguish the phenotypes, it remains unclear whether this explains differences in CVD risk between MONW, MHO, and MAO individuals [68]. One promising avenue of exploration would be to measure various pro- and anti-inflammatory markers, which could provide details about site-specific inflammation beyond the whole-body inflammation reflected by hsCRP [10, 69]. Overall, not only the study of inflammatory markers and adipokines is a promising avenue of research to begin unravelling the MHO phenotype, but also to better understand the MONW phenotype for which little has been studied so far.

9. Risk of Developing Future Complications

Ultimately, the various features discussed above are known to influence the development of obesity-related complications, such as T2D and CVD. The general assumption is that maintaining a healthy lifestyle and maintaining a low body weight will help to minimize an individual’s risk for developing future complications; however, the discovery of MONW and MHO phenotypes suggests that this generalization can be improved. While LH individuals are considered to have the lowest risk of developing future complications, MAO individuals
typically have the highest risk [56, 70]. Understanding the risks of MONW and MHO individuals is therefore crucial for optimally managing their health.

Individuals considered MONW have been reported to present a three to four fold increased risk of developing T2D compared to LH individuals [5] and MHO individuals [49, 71]. Furthermore, MONW individuals are at greater risk for cardiovascular events compared to MHO individuals [49, 71]. Importantly, the risk for MONW has been reported to be comparable to that of MAO subjects [49]. Again, this reinforces that MONW individuals would benefit from an early diagnosis, especially because the risk factors associated with the MONW phenotype are mostly reversible and could be avoided with lifestyle changes [11, 71].

Subjects that are classified as MHO have been found to have an intermediate risk for developing metabolic complications; they appear to have a higher risk than LH individuals, but lower than MAO subjects [1, 5, 19, 32, 63]. For example, Succurro et al. observed that MHO individuals had three to four fold less risk of developing T2D [5] compared to MAO individuals; however, the question remains whether MHO subjects will stay metabolically healthy throughout their life or not. In a recent prospective study looking at the risk of complications in MHO individuals, Appelton et al. reported that the MHO state was stable for 67% of their study participants during a ten-year follow-up period [72]. Moreover, some studies have reported that individuals over the age of 70 [73] and 80 years [45] can be classified as MHO, suggesting that some individuals remain metabolically healthy over time despite their excess fat. This has lead to the suggestion that MHO individuals may be protected or, at minimum, more resistant to the development of metabolic abnormalities related to excess body weight than MAO individuals [1, 45, 71, 74, 75].
Prospective long-term studies need to be realized in order to assess the stability of the MONW and MHO individuals’ health status over time, as well as their associated risks of developing various co-morbidities.

10. Conclusion

As research aims to better understand the heterogeneous obesity phenotype, the MONW, MHO and MAO subgroups are now recognized and discussed in the literature. It is clear that the MONW and the MHO groups display a unique profile with regards to body composition, energy metabolism, inflammation, and adipose tissue morphology, and that they are distinct from the LH and MAO groups.

The identification of these groups of individuals underscores that not all obese people display metabolic abnormalities, and not all lean subjects have a healthy metabolic profile. This paradox reinforces the notion that excess body weight is not the sole determinant of complications like T2D, hypertension, and CVD. Not only will further research in the area generate invaluable new knowledge, but it will also ensure that individuals are correctly assessed for obesity-related complications risks and optimal health management. While body weight and BMI are certainly useful indicators, the inclusion of measurements to assess an individual’s metabolic status appear necessary as we seek to better understand the genetic and molecular basis for obesity.
Chapter II

Rationale, Research Objectives and Hypotheses
RATIONALE

Obesity, as it is classically viewed (i.e., MAO phenotype), predisposes an individual to a wide range of co-morbidities including T2D, CVD, and cancer [58, 76]. Many of these downstream complications are related to the low-grade inflammation that is typically associated with excess fat [77, 78]. The source of this inflammation is from adipose tissue. Indeed, adipose tissue is now seen as more than a reservoir for simply storing excess energy; it is now known to act as an endocrine organ that secretes hundreds of proteins (i.e., adipokines) that regulate whole-body energy homeostasis [76, 79]. In situations of positive energy balance, adipose tissue is remodelled through hypertrophy and hyperplasia of adipocytes [80]. In cases of rapid expansion, the remodelling of adipose tissue is accompanied by hypoxia, adipocyte necrosis, macrophage infiltration, and an imbalance in the secretion of pro- vs anti-inflammatory adipokines. Together, these events promote a low-grade inflammation [58, 77].

As indicated in Chapter I, obesity is a complex heterogeneous phenotype that comprises different subgroups, not all of which face the same health risks. Of particular interest, the MHO group seems to be protected from downstream complications despite their excess fat, while the MONW group develops complications that are usually associated with excess weight. Although these phenotypes are poorly understood, researchers are now focusing their efforts to understand the defining characteristics for each of these groups of individuals and how this may help explain their risks of downstream complications.

One avenue of particular interest is inflammation and circulating adipokines levels. Indeed, the low-grade inflammation associated with excess fat has been suggested to explain why these different subgroups of individuals have different risks for complications [57, 81]. Accordingly, the theory of the “healthy adipose tissue expansion”, as defined by enhanced
recruitment of preadipocytes, adequate vascularization, suitable hypertrophy and hyperplasia, and minimal inflammation [80], could serve as an explanation for the MHO phenotype [57, 77]. However, the link between adipose tissue functions and inflammatory status in the MHO phenotype remains poorly understood. The overall goal of this thesis was to deepen our understanding of the heterogeneous obesity phenotype, and specifically the MHO group, by studying their inflammatory profile.

**RESEARCH OBJECTIVES**

The objective of my thesis was to study the MHO phenotype, a distinct subgroup of obesity, and to characterize the bioclinical and inflammatory profile of these individuals, in comparison to their unhealthy MAO counterparts.

Chapter III had as its main objective to characterize a cohort of LH, MHO, and MAO individuals in order to compare their distinct profile and better appreciate the complexity of the obesity phenotype. Specifically, the objectives of Chapter III were to: 1) advertise and recruit participants into the Diabetes Risk Assessment study, 2) actively conduct study days and collect samples from participants, 3) compile anthropometric and bioclinical measurements and manage the study database, 4) determine the definition criteria for each phenotype (LH, MONW, MHO, and MAO) based on the current literature, 5) create a sub-cohort of age-, sex-, BMI-, and percentage body fat-matched participants on which further tests could be realized, 6) profile the inflammatory profile in the sub-cohort of LH, MHO and MAO groups by measuring a panel of pro- and anti-inflammatory markers, and 7) compare the bioclinical and inflammatory profiles of the MHO individuals to that of the LH and MAO subjects by using the appropriate statistical methods.
Chapter IV provides overall conclusions from my thesis and suggests some future directions that I have identified to improve our understanding of the MHO phenotype.

Finally, Appendix A, despite being not directly related to my thesis research objectives, is included because it provided me with the opportunity to learn the various methodologies required to accomplish my thesis objectives. Appendix A corresponds to a manuscript that is currently under peer-review. This manuscript outlines the relationships between various inflammatory markers and circulating fatty acids (FA) in a cohort of young healthy adults. The realization of this study gave me the opportunity to learn the Bioplex technique to measure circulating inflammatory markers, as well as the management of a large cohort database. It also prompted me to learn numerous statistical methods (e.g., determining the distribution of data, transforming data to achieve normality, looking for and removing outliers, conducting basic associations between 2 variables, building statistical models with covariates, etc.). Importantly, it taught me the process of writing and submitting a peer-reviewed manuscript. All those abilities made the following steps related to my main project, the DRA study, easier and more efficacious.

**HYPOTHESES**

The term *obesity* is widely used to describe a state of excess weight, as defined by a high BMI [47]. The overall hypothesis of my thesis is that distinct subgroups of obesity exist and differ with regards to risk factors, symptoms and, ultimately, downstream complications related to excess weight.
More specifically, my hypothesis was that MHO individuals have a unique bioclinical profile, placing this subgroup of individuals at an intermediate state between the LH and MAO phenotypes. More importantly, I hypothesized that MHO individuals would be characterized by the absence of low-grade inflammation in comparison to the MAO subgroup. This more favourable inflammatory profile may help to explain why MHO individuals are seemingly protected from the downstream complications typically associated with obesity.
Chapter III

Elucidating the inflammatory profile of the Metabolically Healthy Obese
Chapter III Summary

Background. Obesity is generally associated with low-grade inflammation, and this feature is believed to be partly responsible for various downstream complications such as T2D and CVD. A subgroup of obesity, termed the Metabolically Healthy Obese (MHO), seem to be protected against these complications. The absence of low-grade inflammation is one hypothesis to explain the overall healthy metabolic status of MHO individuals compared to Metabolically Abnormal Obese (MAO) individuals.

Objective. The main objective of this chapter was to assess and compare the inflammatory profile of Lean Healthy (LH), MHO and MAO individuals by measuring a panel of circulating pro- and anti-inflammatory markers.

Methods. Thirty participants from the Diabetes Risk Assessment (DRA) study were recruited and classified into LH, MHO or MAO groups (n=10/group) based on their adiposity status and metabolic status. Various anthropometric (including body composition by BOD POD™) and bioclinical measurements (including circulating markers of glucose and lipid metabolism by LifeLabs Medical Laboratory Services) were performed. Moreover, a panel of pro- and anti-inflammatory markers were measured in plasma using immunoassay methodologies (ELISA and Bioplex Bead assays). Analysis of variance (ANOVA) and student t-tests were used to assess significance between groups (p<0.05).

Results. The anthropometric markers revealed that LH, MHO and MAO groups were matched for age and sex, while the two obese groups were also matched for BMI and percentage body fat. MHO individuals had Total-c and LDL-c levels significantly lower compared to the MAO group. In contrast, the MHO individuals were similar to the MAO subjects and had significantly lower levels of HDL-c and TG compared to the LH group, while they had an intermediate value for
Total-c/HDL-c ratio. The MHO group did not differ from the MAO group when looking at fasting glucose and fasting insulin values, HbA1c, and HOMA-IR. MHO individuals had lower levels of hsCRP and IL-6 compared to MAO subjects. Also, MHO subjects had similar levels of HMW adiponectin to the MAO group, but levels of PDGF-ββ were intermediate to those of the LH and MAO groups.

**Conclusion.** Overall, MHO individuals had a distinct lipid and inflammatory profile that was more favourable than their unhealthy MAO counterparts.
1. Background

Obesity is now recognized as a chronic low-grade inflammatory state [77] that plays an important role in the development of complications such as insulin resistance, hypertension, CVD and T2D [58, 76]. The role of adipose tissue as an active endocrine organ seems to be central to the subclinical inflammation observed with excess weight [64]. Indeed, adipose tissue is able to regulate energy homeostasis [76, 79]. In situations of excessive caloric intake, adipose tissue undergoes remodelling through hypertrophy and hyperplasia of the adipocytes [80]. In case of rapid expansion, the remodelling can be accompanied by adipose tissue hypoxia and necrosis, macrophage infiltration, and imbalance between the secretion of pro- and anti-inflammatory adipokines, hence promoting a low-grade inflammation [58, 77]. Despite having a similar BMI as MAO individuals, MHO individuals appear to be protected from the various metabolic disturbances generally associated with excess body weight, such as insulin resistance and dyslipidemia [15]. One potential explanation for this is that MHO individuals may have an absence of low-grade inflammation [65, 82, 83].

Karelis et al. previously studied inflammatory markers in a cohort of post-menopausal sedentary Caucasian women (22 women in the MHO group as well as in the MAO group) [52]. Despite a similar percentage body fat to MAO women, MHO women had significantly lower levels of the whole-body inflammatory marker hsCRP, as well as a significantly lower level of the inflammation-sensitive protein α-1 anti-trypsin. Another study by Klöting et al. looked at circulating adipokines as well as markers of inflammation in adipose tissue in a cohort of 60 individuals where MHO individuals (n=30) were matched to MAO subjects (n=30) based on age, sex and percentage body fat [57]. The authors reported that MHO had significantly lower circulating levels of various inflammatory markers (i.e., CRP, progranulin, chemerin, and
retinol-binding protein-4) compared to MAO subjects, as well as a significantly lower number of macrophages in their visceral adipose tissue. This study demonstrated that the MHO group have less inflamed visceral adipose tissue and lower levels of pro-inflammatory markers in the blood compared to MAO subjects. Taken together, these studies suggest that MHO have a lower level of chronic inflammation compared to MAO subjects, independent of total body fat.

To our knowledge, no study has previously looked at a wide panel of pro- and anti-inflammatory markers in MHO and MAO individuals. Examining circulating adipokines in addition to hsCRP will provide invaluable insight to help unravel the inflammatory profiles of MHO and MAO subjects. Indeed, hsCRP is commonly used in clinical practice to measure whole-body inflammation; however, it does not provide information regarding which inflammatory pathways are being modulated [84, 85]. In contrast, specific cytokines and chemokines can provide clues regarding which biological processes and functions are affected [76]. This is of crucial importance in order to relate circulating markers to cellular dysregulation in adipose tissue and other organs [86, 87].

In order to better understand the inflammatory status of MHO and MAO subjects, we recruited 30 subjects from the Diabetes Risk Assessment (DRA) study and classified them as LH, MHO, and MAO according to anthropometric and bioclinical measurements. The inflammatory profile of each group was studied by measuring a panel of pro- and anti-inflammatory markers in order to determine if the MHO individuals have a more favourable profile than MAO subjects. A deeper understanding of the MHO group will help to better understand why these individuals appear to be protected from obesity-related complications. Furthermore, this work will help lead to more personalized and efficacious treatments (e.g.,
dietary recommendations, physical activity strategies, etc.) for different subgroups of obese subjects.

2. Materials and Methods

Study population

Participants from the Diabetes Risk Assessment (DRA) study were used for the present investigation. The DRA study is a human clinical study conducted at the University of Guelph in collaboration with family physicians in the Guelph Family Health Team and the Public Health Agency of Canada (Clinical Trial Registration number NCT01884714). Recruitment began in September 2011 and, as of now, 65 participants have taken part in the DRA Study. Participants were recruited from Guelph and surrounding areas using various methods including study posters, recruitment sessions at the Diabetes Care Guelph Clinic, and direct invitation by the physician involved in the DRA study. Individuals were excluded if they met one of the following criteria 1) below 35 or above 70 years, 2) presenting a diagnosis of acute or chronic autoimmune inflammatory disease, infectious diseases, viral infection, and/or cancer, and 3) regular alcohol consumption exceeding 2 drinks/day (1 drink = 10 g alcohol). Participant eligibility was assessed during a phone call. Forty-eight hours prior to the study visit, participants were asked to avoid rigorous exercise, over-the-counter medication, dietary supplements (i.e., vitamins and herbal supplements), and alcohol consumption. Before the study visit, participants were required to fast overnight. The study was conducted at the Human Nutraceutical Research Unit at the University of Guelph. All participants signed a consent form the morning of the study day. The research protocol was approved by the University of Guelph Human Research Ethics Board (REB#10AP033).
Anthropometric and bioclinical measurements

Anthropometric measures (height, weight, waist circumference and hip circumference) and body composition (body weight, fat mass (% and kg) and lean mass (% and kg) were determined using the air displacement plethysmography method (BOD POD™, Cosmed, California, USA) located in the Body Composition and Metabolism Laboratory at the University of Guelph. BMI was calculated by dividing body mass (kg) by height (m²). The waist-to-hip ratio was determined by dividing waist by hip circumference (cm).

Blood pressure was measured in duplicate in resting participants using an automated blood pressure monitor (Intellisense, OMRON Healthcare, Bannockburn, Illinois, USA).

Fasting serum samples were collected from each participant in serum-separating tubes (SST tubes with preservation additive, clot activator and serum separation gel), which was allowed to clot at room temperature for 30 min, followed by 15 min of centrifugation at 1200g, and stored at 4°C until pick-up by Lifelabs Medical Laboratory Services (Kitchener, ON, Canada) for the measurement of blood TG, Total-c, LDL-c, HDL-c, hsCRP, and glucose according to standard procedures. For measurement of insulin levels, fasting serum samples were collected following the same procedure except that it was transferred into a freezing tube and store at -20°C overnight before being shipped frozen to Lifelabs Medical Laboratory Services. Also, fasting plasma samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes stored at 4°C after collection and until pick-up by Lifelabs Medical Laboratory Services for measurements of HbA1c according to standard procedures. Estimates of insulin resistance (HOMA-IR) and β-cell function (HOMA%B) were determined using the HOMA calculator [88].
For the measurement of inflammatory markers, fasting serum samples were collected in serum tubes (SST tubes with preservation additive, clot activator and serum separation gel) which was allowed to clot at room temperature for 30 min, followed by 15 min of centrifugation at 1200g, aliquoted into individual tubes, and stored at -80°C until processing.

**Group classification**

All participants of the DRA Study cohort (n=65) were classified based on their adiposity and metabolic status (Box 2). Specifically, adiposity status was determined with BMI, where lean (BMI < 28 kg/m² for males, < 24 kg/m² for females) or obese (BMI ≥ 28 kg/m² for males, ≥ 24 kg/m² for females) groups were created. Based on the set of criteria suggested by Karelis et al. [15], an individual was considered to have a healthy status if 3 or more criteria were met (HDL-c > 1.0 mmol/L for males, > 1.3 mmol/L for females; TG < 1.7 mmol/L without use of lipid-lowering drug, Total-c < 5.2 mmol/L, LDL-c < 2.6 mmol/L, HOMA-IR < 1.95 without use of antidiabetic drug) while an individual was considered unhealthy if they met 2 or less of the aforementioned criteria.

<table>
<thead>
<tr>
<th>Adiposity status criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean</td>
</tr>
<tr>
<td>Obese</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Metabolic status criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
</tr>
<tr>
<td>Unhealthy</td>
</tr>
</tbody>
</table>

Box 2: Summary of the identification criteria used in the DRA study cohort. The LH, MONW, MHO and MAO groups have been created using a combination of the adiposity status (using the BMI as suggested by [39]) and the metabolic status (using a set of metabolic criteria as suggested by [15]).

From the DRA study cohort, a subset of 30 individuals were selected to create the LH (n=10), MHO (n=10) and MAO (n=10) groups. MHO individuals were matched with MAO individuals by age, sex, BMI and percentage body fat. Subsequently, LH subjects were matched...
by age and sex to the MHO and MAO cohorts formed. Noteworthy, not enough participants were classified as MONW to permit the creation of a MONW group.

**Inflammatory markers measurements**

A fasting serum sample from each participant was used to measure pro- and anti-inflammatory markers. Interleukin-10 (IL-10), monocyte chemotactic protein (MCP-1, also known as CCL2), and tumour-necrosis factor (TNF-α) were measured using immunoassay kits, according to the manufacturer’s instructions (LEGEND Max pre-coated ELISA, BioLegend, San Diego, California, USA). HMW adiponectin was measured using an immunoassay kit, according to the manufacturer’s instructions (Quantikine ELISA, R&D Systems, Minneapolis, Minnesota, USA). Briefly, serum samples (50 µL, in duplicate) were first incubated at room temperature with the capture antibody of interest and then subsequently incubated with detection antibody and an avidin-horseradish peroxidase solution to permit absorbance reading. Plates were read at 450 and 570 nm using a plate reader (SynergyMX plate reader, Biotek, Winooski, Vermont, USA). The wavelength obtained at 570 nm was subtracted from the one obtained at 450 nm in order to minimise the background noise as recommended in the manufacturer’s protocol. Concentrations were determined using the Gen 5 Data analysis software (Version 2.0).

Interleukin-6 (IL-6), interleukin-1 receptor antagonist (IL-1Ra), interferon-γ (IFN-γ), regulated upon activation normal T-cell expressed and secreted (RANTES, also known as CCL5), platelet derived growth factor-ββ (PDGF-ββ), and interferon-γ inducible protein 10 (IP-10) were measured by multiplex bead immunoassay (Bio-Plex 200, Bio-Rad, Mississauga, ON, Canada). Serum samples (30 µL, in duplicate) were diluted 1:4 with sample diluent and the assay was run according to the manufacturer’s instructions. Beads were read using the Bio-Plex
suspension array system and concentrations determined with Bio-Plex Manager software (Version 6.0).

**Table 3** summarizes the inflammatory markers studied in this cohort and provides an overview of their biological roles and origin of production in the body.

**Statistical analysis**

All data were analyzed using the GraphPad Prism 5 software. Considering our population size, a non-parametric ANOVA Kruskal-Wallis test was used to measure significance between the three groups. When significance was observed, a post-hoc non-parametric Mann Whitney test was used to compare two groups. A p < 0.05 was considered statistically significant.
Table 3: Summary of the inflammatory markers measured in the DRA study cohort. Pro- and anti-inflammatory markers, their acronyms, as well as an overview of their site of production and roles.
3. Results

Characteristics of the groups

General characteristics of the study population are outlined in Table 4. Briefly, the anthropometric markers revealed that LH, MHO and MAO groups were adequately matched for age (mean age of 49 ± 2 yrs) and sex (3 men and 7 women per group), while the two obese groups were also matched for BMI (MHO: 30.6 ± 1.11 and MAO: 33.0 ± 1.9 kg/m²) and percentage body fat (MHO: 39.8 ± 2.4 and MAO: 39.3 ± 2.4 %). Moreover, the obese groups were not significantly different for waist circumference (MHO: 98 ± 3 and MAO: 104 ± 4 cm), hip circumference (MHO: 109 ± 2 and MAO: 113 ± 4 cm) and waist-to-hip ratio (MHO: 0.90 ± 0.03 and MAO: 0.92 ± 0.02).

We also examined circulating lipid markers in the three groups. Total-c (MHO: 4.26 ± 0.32 and LH: 4.43 ± 0.30 vs MAO: 5.34 ± 0.23 mmol/L) and LDL-c (MHO: 2.39 ± 0.30 and LH: 2.52 ± 0.25 vs MAO: 3.27 ± 0.19 mmol/L) were similar between the MHO and LH groups, and significantly lower compared to the MAO group. In contrast, the MHO and MAO groups were similar and significantly lower for HDL-c (MHO: 1.17 ± 0.12 and MAO: 1.04 ± 0.05 vs LH: 1.57 ± 0.08 mmol/L) and TG (MHO: 1.54 ± 0.33 and MAO: 2.26 ± 0.23 vs LH: 0.77 ± 0.05 mmol/L) compared to the LH group. Accordingly, the MHO group had an intermediate value for Total-c/HDL-c ratio (LH: 2.85 ± 0.16, MHO: 3.81 ± 0.22, MAO: 5.17 ± 0.18).

The MHO group did not differ from the MAO group with respect to fasting glucose and fasting insulin values, HbA1c, HOMA-IR and HOMA%B.
<table>
<thead>
<tr>
<th>Parameters</th>
<th>LH Mean ± SEM</th>
<th>MHO Mean ± SEM</th>
<th>MAO Mean ± SEM</th>
<th>ANOVA LH vs MHO</th>
<th>ANOVA LH vs MAO</th>
<th>ANOVA MHO vs MAO</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthropometric measurements</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of subjects</td>
<td>10 (3 men, 7 women)</td>
<td>10 (3 men, 7 women)</td>
<td>10 (3 men, 7 women)</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>51 ± 3</td>
<td>50 ± 4</td>
<td>48 ± 2</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>61.9 ± 2.8</td>
<td>86.2 ± 3.4</td>
<td>92.7 ± 6.2</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167 ± 3</td>
<td>168 ± 3</td>
<td>167 ± 3</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.1 ± 0.6</td>
<td>30.6 ± 1.1</td>
<td>33.0 ± 1.9</td>
<td>NS</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>77 ± 3</td>
<td>98 ± 3</td>
<td>104 ± 5</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>96 ± 1</td>
<td>109 ± 2</td>
<td>113 ± 4</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.80 ± 0.02</td>
<td>0.90 ± 0.03</td>
<td>0.92 ± 0.02</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>27.4 ± 2.7</td>
<td>39.8 ± 2.4</td>
<td>39.3 ± 2.4</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>16.7 ± 1.6</td>
<td>34.1 ± 2.4</td>
<td>36.4 ± 3.4</td>
<td>NS</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lean mass (%)</td>
<td>72.6 ± 2.7</td>
<td>60.2 ± 2.4</td>
<td>60.8 ± 2.4</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>45.1 ± 3.1</td>
<td>52.0 ± 3.2</td>
<td>56.2 ± 4.4</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bioclinical measurements</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>118 ± 4</td>
<td>128 ± 5</td>
<td>128 ± 4</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>75 ± 3</td>
<td>82 ± 2</td>
<td>82 ± 2</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Total-c (mmol/L)</td>
<td>4.43 ± 0.30</td>
<td>4.26 ± 0.32</td>
<td>5.34 ± 0.23</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>LDL-c (mmol/L)</td>
<td>2.52 ± 0.25</td>
<td>2.39 ± 0.30</td>
<td>3.27 ± 0.19</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>HDL-c (mmol/L)</td>
<td>1.57 ± 0.08</td>
<td>1.17 ± 0.12</td>
<td>1.04 ± 0.05</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total-c/HDL ratio</td>
<td>2.85 ± 0.16</td>
<td>3.81 ± 0.22</td>
<td>5.17 ± 0.18</td>
<td>&lt;0.0001</td>
<td>NS</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>0.77 ± 0.05</td>
<td>1.54 ± 0.33</td>
<td>2.26 ± 0.23</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>4.5 ± 0.2</td>
<td>5.0 ± 0.1</td>
<td>5.3 ± 0.2</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Fasting insulin (pmol/L)</td>
<td>37 ± 17</td>
<td>64 ± 9</td>
<td>118 ± 22</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.37 ± 0.07</td>
<td>5.72 ± 0.08</td>
<td>5.73 ± 0.08</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.65 ± 0.28</td>
<td>1.18 ± 0.16</td>
<td>2.19 ± 0.42</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>HOMA%B</td>
<td>93.7 ± 33.1</td>
<td>105.9 ± 10.1</td>
<td>140.3 ± 17.9</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

**Table 4: DRA study population characteristics.** Data represented as mean ± SEM. BMI, body mass index; BP, blood pressure; Total-c, total-cholesterol; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; TG, triglycerides; HbA1c, glycosylated haemoglobin; HOMA-IR, homeostatic model assessment for insulin resistance; HOMA%B, homeostatic model assessment for β-cell function. Non-parametric one-way ANOVA Kruskal-Wallis followed by post-hoc Mann Whitney tests have been used to determine the significance (p<0.05).
**Inflammatory status**

Table 5 outlines the mean circulating concentrations of the inflammatory markers measured in the DRA study cohort subjects. TNF-α, MCP-1 and IL-10 levels were below the detection threshold in our cohort (data not shown). Amongst the three groups, no statistical differences were observed for IL-1Ra, IFN-γ, and RANTES.

![Table 5](data:image/png;base64,iVBORw0KGgoAAAANSUhEUgAAAIgAAAAHCAIAAAD5AFkdAAAABGdBTUEAALGPC/xhBQAAAAB3RkJXAgIbGwAAACBklEQtFADgBAAABl6jJkAAAA1XRFWHRD9AAAA1SURBVHgFzCgY80QzDR8AgA37i8BAAAABJRU5ErkJggg==)

**Table 5: Mean circulating concentration of inflammatory markers in fasting serum of the DRA study groups.**

Data represented as mean ± SEM. hsCRP, high sensitive C reactive protein; IL-6, interleukin-6; IFN-γ, interferon γ; IP-10, interferon-γ inducible protein 10; PDGF-ββ, platelet-derived growth factor ββ; RANTES, regulated upon activation normal T-cell expressed and secreted; HMW adiponectin, high molecular weight adiponectin; IL-1Ra, interleukin-1 receptor antagonist. Non-parametric one-way ANOVA Kruskal-Wallis followed by post-hoc Mann Whitney tests have been used to determine the significance (p<0.05).

As shown in Figure 3, MHO individuals had hsCRP levels statistically lower than MAO subjects (MHO: 1.75 ± 0.45 and LH: 0.76 ± 0.19 vs MAO: 5.35 ± 1.99 mg/L). This profile was similarly observed for IL-6, where the MHO group resembled the LH group (MHO: 1.37 ± 0.31 and LH: 1.04 ± 0.17 vs MAO: 2.52 ± 0.53 pg/mL). When looking at PDGF-ββ, the MHO individuals had an intermediate level between the LH and MAO groups (LH: 7373 ± 621, MHO: 8751 ± 793, MAO: 9933 ± 468 pg/mL). A similar trend was observed for the pro-inflammatory...
marker IP-10, where MHO individuals had an intermediate level compared to the LH and MAO groups; however, this was only borderline significant (p=0.07, Table 5). In contrast, MHO subjects had similar HMW adiponectin levels to MAO subjects, which was considerably less than LH individuals (MHO: 2794 ± 412 and MAO: 2752 ± 529 vs LH: 6914 ± 1382).

Figure 3: Mean concentration of inflammatory markers in fasting serum of the DRA study groups. (A) hsCRP, high sensitive C reactive protein; (B) IL-6, interleukin-6; (C) PDGF-ββ, platelet-derived growth factor ββ; and (D) HMW adiponectin, high molecular weight adiponectin in Lean Healthy (LH, n=10), Metabolically Healthy Obese (MHO, n=10) and Metabolically Abnormal Obese (MAO, n=10) groups. Non-parametric one-way ANOVA Kruskal-Wallis followed by post-hoc Mann Whitney tests have been used. Bars sharing different letters are statistically different (p<0.05).

4. Discussion

The identification of the MHO phenotype has raised questions about the wide-spread belief that excess body fat is consistently associated with adverse metabolic complications [77]. In contrast to MAO individuals who have a dysfunctional adipose tissue associated with low-grade inflammation [47], it has been hypothesized that MHO individuals may show no signs of this low-grade inflammatory state due to a “healthy” adipose tissue [70, 80].
In the present study, we observed that MHO individuals had lower levels of hsCRP and IL-6 compared to MAO subjects. The HMW adiponectin level in MHO subjects was not significantly different to that observed in the MAO group, while MHO subjects had intermediate levels of PDGF-ββ compared to LH and MAO groups. The same trend was observed for the pro-inflammatory marker IP-10, where MHO individuals had an intermediate level compared to the LH and MAO groups; however, this was only borderline significant (p=0.07). Overall, the results of the current investigation indicate that middle-aged individuals characterized as MHO have a more favourable inflammatory profile than individuals classified as MAO, despite a similar level of adiposity.

Various concentrations of circulating anti-inflammatory (IL-1Ra, IL-10, and HMW adiponectin) and pro-inflammatory markers (TNF-α, IFN-γ, MCP-1/CCL2, IL-6, RANTES/CCL5, PDGF-ββ, and IP-10) were measured (Table 3), but some markers were undetected (TNF-α, MCP-1 and IL-10) in the present population. Since the goal of this study was to assess low-grade inflammation and one of our exclusion criteria included the presence of an acute and/or chronic inflammatory disease, it was not surprising that some markers could not be detected in our study cohort. Nevertheless, our data confirmed previous findings that MHO individuals have lower levels of hsCRP and IL-6 compared to MAO individuals with a similar percentage body fat [52, 57]. Of interest, we noted that the markers measured in the current study that are secreted predominantly by adipose tissue were not statistically different between groups. In fact, the major differences between MHO and MAO groups were observed with hsCRP and IL-6 levels. This is not surprising given that hsCRP is produced mainly by the liver in response to circulating IL-6 concentrations [76]. However, the fact that inflammatory markers derived from adipose tissue did not differ significantly between MHO and MAO subjects suggests that
this may not be the origin for differences between these two subgroups of obese individuals. Rather, it is tempting to speculate that the liver could be an important player in the aetiology of the MHO phenotype.

Few studies have looked closely at liver function in the context of the MHO phenotype. Messier et al. measured plasmatic concentrations of hepatic enzymes in a population of a hundred post-menopausal women, where MHO women were identified as having a BMI > 30kg/m² and an insulin-sensitivity value in the upper quartile as determined by OGTT-derived Matsuda index [34]. Although MHO and MAO women were matched for age and BMI, the MHO group had significantly lower levels of the hepatic enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST) and γ-glutamyltransferase (GGT), as well as a lower fatty liver index which is a predictor of hepatic steatosis [89]. Similar results were recently reported by Hong and colleagues in a larger Asian cohort of 16,000 participants aged 30 to 45 years and classified as MHO using a cut-off of 25 kg/m² for BMI and the presence of ≥ 4 healthy criteria based on NCEP-ATP III criteria [55]. Taken together, the results of these studies suggest that the MHO phenotype could be characterized by lower hepatic insulin resistance and reduced ectopic fat deposition in the liver, as shown by decreased levels of circulating hepatic enzymes [90].

When considering HMW adiponectin, we were surprised to observe that the MHO group had similar circulating levels compared to MAO individuals. Moreover, these levels were significantly lower than the ones observed in the LH group. This result is discordant with literature as MHO individuals are usually assumed to be more insulin sensitive and have a higher level of adiponectin than MAO individuals [22, 29, 67, 91, 92]. However, the result we observed in our study could be explained by the criteria used to classify individuals. Based on the
classification criteria used in our study (set of criteria as suggested by [15], Box 2), we have characterized MHO individuals primarily based on their lipid profile rather than on insulin sensitivity parameters (as shown by no statistical differences between MHO and MAO in terms of fasting glucose, insulin, HbA1c, HOMA-IR and HOMA%B). As adiponectin is a molecule with insulin-sensitizing properties [64], it is therefore not unexpected that the level found in the MHO group did not differ from the one observed in the MAO group. Despite not differing with regards to insulin sensitivity, the MHO and MAO subjects in our cohort differed with respect to their lipid and inflammatory profiles, suggesting that MHO individuals have an overall “healthier” status. When looking at circulating lipids, the MHO group had an intermediate profile between the LH and MAO groups, concordant with results observed by Marini and colleagues in their cohort of middle-aged women [32]. As highlighted in Chapter I, the use of different criteria to identify MHO subjects seems to suggest that different aetiologies may be associated with the same phenotype, i.e., some MHO individuals may be characterized by a preserved insulin sensitivity while others may be characterized by a favourable circulating lipid profile [71].

The results observed in this study contribute to the growing body of evidence reinforcing the urgent need for a set of criteria to define the MHO phenotype [15, 38, 93-97]. Despite the recent increase in studies examining the MHO phenotype, there is still no consensus regarding the criteria to identify these individuals. While the present study revealed that the criteria used to define the metabolic status are important when studying the MHO profile, it is also true for measures of adiposity status. BMI has been questioned in terms of its sensitivity to robustly reflect a person’s adiposity [40] and to predict health risks associated with excess weight [98]. To circumvent this limitation we used BMI and the sex-specific cut-offs suggested by Shah and
Braverman [39]. These values were found to maximize the specificity and sensitivity of using BMI to predict adverse health risks. Importantly, using this approach resulted in subjects deemed “overweight” being merged with subjects considered “obese”. However, despite using this approach, the validity of BMI to predict obesity-related complications can be questioned in the context of the MHO phenotype. Indeed, according to the results observed in the present study, MHO subjects had a more favourable profile than MAO subjects despite being matched for BMI. This suggests that MHO subjects appear to have a reduced risk for obesity-related complications, reinforcing that BMI is not the sole determinant by which to assess health risks. Of interest, other commonly used anthropometric measurements, namely waist circumference, hip circumference as well as the waist-to-hip ratio, did not differentiate MHO from MAO individuals in our cohort; thus their use for predicting health risk related to fat distribution appear to be limited [34].

Overall, the results of the present investigation suggest that MHO individuals, despite the same level of adiposity than the MAO subjects, present a distinct bioclinical profile as observed by measuring circulating lipids. It was also found that MHO individuals have an intermediate level of inflammation compared to the LH and MAO groups, reinforcing the notion of a heterogeneous obesity phenotype.
Chapter IV

Overall Conclusions and Future Directions
OVERALL CONCLUSIONS AND FUTURE DIRECTIONS

The general objective set for this thesis was achieved. Specifically, I demonstrated that, as hypothesized, MHO individuals have a distinct and more favourable lipid and inflammation profile compared to their unhealthy MAO counterparts. This illustrates the complexity and heterogeneity of the obesity phenotype.

Chapter I provided a global overview of the obesity phenotype and highlighted differences between the MONW, MHO and MAO groups. This thorough literature review outlined several key aspects demonstrating that the MONW and MHO groups are distinct phenotypes in terms of body composition, energy metabolism and adipose tissue functions. In addition, future studies should look at the lifestyle of these individuals; specifically dietary habits and physical activity levels, in order to further deepen our knowledge of the MONW and MHO groups. Although it has a heritable component, obesity is considered to be a lifestyle disease where nutrition and physical activity are key environmental factors that can promote or prevent weight gain, thus influencing an individual’s health status [73]. Accordingly, it is important to examine these two aspects in MONW and MHO individuals. A physically active lifestyle combined with a healthy and balanced diet may be linked to the MHO phenotype, while the opposite could be linked to the MONW phenotype. As such, knowledge of an individual’s lifestyle represents essential information that can help to better understand the different risks associated to the MONW, MHO and MAO.

Chapter III tested the hypothesis that MHO individuals have lower levels of inflammation compared to MAO individuals. As expected, the results obtained through the DRA study showed that MHO individuals have an inflammatory status that is intermediate to the LH and MAO groups. While we observed significant results with ten participants per group, it will be valuable
and necessary to increase the size of the DRA cohort in order to not only gain statistical power, but to also be able to create a group of MONW individuals. Measuring the inflammatory markers of the MONW individuals would be valuable to explore why, despite relatively normal BMI, they present similar co-morbidities as MAO individuals. Despite their characteristically lower body mass compared to the MAO individuals, MONW individuals have complications generally associated with obesity (as outlined in Chapter I). One hypothesis to explain their condition could be the presence of a higher level of percentage visceral fat. Therefore, future studies should explore their body fat distribution and anthropometric measurements, as well as their inflammatory profile, in order to better understand the differences between MONW, MHO and MAO individuals.

More broadly, future directions could include the exploration of insulin sensitive organs such as the liver (as previously mentioned in Chapter III), and adipose tissue. To provide insight on downstream complications of obesity, a closer look at adipose tissue function could be done using various approaches. Histological analyses would permit exploration of the structure of the adipose tissue. As most studies are actually realized using indirect measurements, histology work on various fat depots will allow the investigation of the size and number of adipocytes, and provide insight regarding hypertrophy and hyperplasia in adipose tissue. Moreover, histological approaches would also permit the study of macrophage infiltration, hypoxia and necrosis, which would provide information regarding adipose tissue remodelling, vascularisation, and inflammation. Also, gene expression profiling would give indications of genes that are up- or down-regulated in adipose tissue and would provide insight into pathways associated with the MONW, MHO, and MAO phenotypes. From there, mechanistic studies could be realized using animal models developed to represent the MONW and MHO phenotypes [70, 99]. Despite the
number of studies that have sought to better understand and clinically characterize the MHO, MONW, and MAO phenotypes, the molecular mechanisms underlying these specific phenotypes remain unknown. Together, such explorations will provide useful information to better understand the molecular differences between the MONW, MHO and MAO groups, complementing the clinical approach currently favoured in the field of obesity research.

In conclusion, this thesis demonstrated that obesity is a complex and heterogeneous phenotype, as observed by the existence of the MONW, MHO and MAO groups. In addition, the work realized during my thesis has shown that MHO is a distinct subgroup of individuals, who show a level of inflammation that is intermediate to LH and MAO individuals. Overall, this thesis has demonstrated that classifying subjects using solely their “weight on the scale” is insufficient to capture their risk for potential health complications. As such, it is paramount to include the assessment of a person’s metabolic status in order to more accurately assess their risk for health complications and develop personalized health management strategies for distinct subgroups of individuals.
REFERENCES


Distinct associations between individual plasma fatty acids and markers of inflammation in young healthy adults: a cross-sectional study.

Maude Perreault, Kaitlin Roke, Alaa Badawi, Daiva E. Nielsen, Ahmed El-Sohemy, Salma A. Abdelmagid, David W.L. Ma, and David M. Mutch.

Lipids, Submitted August 2013.
Appendix A Summary

Inflammation is a recognized risk factor for the development of chronic diseases, such as type 2 diabetes and atherosclerosis. Evidence suggests that individual fatty acids (FA) may have distinct influences on inflammatory processes. The goal of this study was to conduct a cross-sectional analysis to examine the associations between circulating FA and markers of inflammation in a population of young healthy Canadian adults. FA, high-sensitivity C-reactive protein (hsCRP), and cytokines were measured in fasted plasma samples from 965 young adults (22.6 ± 0.1yr). Gas chromatography was used to measure FA. The following cytokines were analyzed with a multiplex assay: Regulated Upon Activation Normal T-cell Expressed and Secreted (RANTES/CCL5), Interleukin 1-Receptor Antagonist (IL-1Ra), Interferon-γ (IFN-γ), Interferon-γ Inducible Protein 10 (IP-10), and Platelet-derived Growth Factor β (PDGF-ββ). Numerous statistically significant associations (p<0.05, corrected for multiple testing) were identified between individual FA and inflammatory markers using linear regression. Myristic (14:0), palmitic (16:0), palmitoleic (16:1), and dihomo-γ-linolenic (20:3n6) acids were positively associated with all six inflammatory markers studied. In contrast, stearic acid was inversely associated with hsCRP and RANTES, and linoleic acid was inversely associated with hsCRP, RANTES and PDGF-ββ. In conclusion, our results indicate that specific FA have distinct associations with various inflammatory markers. This study highlights the importance of considering the distinct biological roles of individual FA on the regulation of inflammation.
1. Introduction

Fatty acids (FA) are able to regulate inflammation through various processes, including regulation of cytokine transcription [1] and eicosanoid production [2]. Typically, FA are discussed according to their degree of desaturation, i.e. saturated FA (SFA), monounsaturated FA (MUFA), polyunsaturated FA (PUFA); however, this has inadvertently contributed to the widespread misconception that all FA within a group function similarly. In other words, it is commonly stated that SFA are pro-inflammatory and PUFA are anti-inflammatory; however, emerging evidence suggests that individual FA within a given class have distinct associations with inflammation [3, 4].

Clinical trials have shown that an individual’s inflammatory status can be modulated by dietary FA [5, 6], while cross-sectional analyses have identified relationships between individual blood FA and markers of inflammation [3, 7]. However, past research has tended to focus on older adults [8], postmenopausal women [9], and at-risk populations [6, 10]. Few studies have examined associations between FA and inflammation in young adults. This is relevant given that low-grade inflammation is a recognized risk factor for the development of chronic diseases, such as type 2 diabetes and atherosclerosis [1, 11].

Indeed, the production of pro-inflammatory molecules such as acute-phase proteins and cytokines promotes tissue damage, vascular dysfunction, and dysregulation of glucose and lipid homeostasis [12]. In the vast majority of studies high-sensitivity C-Reactive Protein (hs-CRP) is used as a clinical marker for whole-body inflammation. While hsCRP is widely used, it does not provide insight regarding which inflammatory pathways are being regulated [13]. Therefore, examining circulating cytokines and chemokines, in addition to hsCRP, may provide greater insight into which inflammatory processes are being regulated in a particular context.
In the present cross-sectional analysis we have examined the relationship between individual FA and several markers of inflammation in young healthy Canadian adults. Assessing these relationships in young adults will provide new information regarding the contribution of individual FA towards modulating inflammation prior to the onset of chronic disease.

2. Methods

Study Population

Subjects participating in the Toronto Nutrigenomics and Health study were used for the present investigation [14]. All participants were between the ages of 20-29 yrs and recruited from the University of Toronto campus between October 2004 and June 2009. From the initial 1005 participants available, 40 subjects were excluded from the analysis because they met one of the following criteria: 1) a body mass index > 35 kg/m^2, 2) plasma hsCRP > 10 mg/L, 3) plasma hsCRP > 3mg/L and a recorded inflammatory event (e.g. a piercing or a fever) 2 weeks prior to providing a blood sample, or 4) were smokers. The final cohort (n=965) consisted of both men (n=282) and women (n=683). The study protocol was approved by the Research Ethics Boards at the University of Toronto and the University of Guelph. Written, informed consent by the subjects was given prior to their participation in the study.

Anthropometric and clinical measurements

Height, weight, waist circumference and body mass index (BMI) were measured, as previously described [14]. Following a 12hr overnight fast, plasma samples were collected and analyzed by a LifeLabs Medical laboratory (Toronto, ON, Canada). Markers of glucose and lipid homeostasis (e.g., glucose, insulin, triglycerides, total- and HDL-cholesterol) and inflammation (hsCRP) were measured using standard procedures.
**Analysis of plasma FA using gas chromatography**

FA content was determined from fasting blood samples using gas chromatography, as previously described [15]. For the present work we examined FA levels using two methods: relative percentage (% of total FA) and absolute concentration (µg/mL). Only FA systematically detected in all subjects were considered for the current analyses. Relative FA values were calculated as a % of total FA and are reported as % FA ± SEM. Absolute FA values were calculated by comparing individual FA peaks to the C17:0 internal standard and are reported as µg FA per mL plasma ± SEM. Absolute FA values were also used to determine the molar concentration (µM) for each FA.

**Analysis of plasma cytokines using multiplex assay**

A multiplex bead assay analysis was performed using a Bio-Plex-200 instrument (Bio-Rad, Mississauga, ON, Canada). As previously described, we first used a commercially available kit to examine 27 common cytokines / chemokines in a subset of 70 individuals in order to determine which inflammatory markers could be consistently detected in our study population [12]. Since our population consisted of young healthy adults, it was expected that many cytokines would be below the detection threshold of our analytical platform. To avoid drawing erroneous conclusions on the relationship between FAs and inflammation, we chose to examine only the five cytokines that were consistently detected in all subjects. The five cytokines measured included Regulated Upon Activation Normal T-cell Expressed and Secreted (RANTES or CCL5), Interleukin 1-Receptor Antagonist (IL-1Ra), Interferon-γ (IFN-γ), Interferon-γ Inducible Protein 10 (IP-10), and Platelet-derived Growth Factor β (PDGF-ββ). Briefly, RANTES is a chemotactic factor that recruits macrophages and leukocytes to sites of inflammation [16]. IL-1Ra is a cytokine that inhibits the inflammatory effects of IL-1α and IL-1β
IFN-γ is a pro-inflammatory cytokine involved in various immunostimulatory responses [18]. IP-10 is a pro-inflammatory cytokine produced in response to IFN-γ [18]. Lastly, PDGF-ββ is a pro-inflammatory growth factor that is involved in angiogenesis [19].

Plasma samples (30 uL) were diluted 1:4 with sample diluent and the assay was run according to the manufacturer’s instructions. Beads were detected using the Bio-Plex suspension array system and concentrations (pg/ml) determined with Bio-Plex Manager software (Version 6.0). Analytical reproducibility was assessed by calculating intra-assay coefficients of variability (CV) (as the average of three standards within each analytical run), and resulted in CV ranges <9% for all cytokines except IFN-γ, where intra-assay CV were 11%. Additionally, inter-assay CV for all five cytokines, calculated across fifteen assays run on different days, were <5%. Each plate was individually normalized according to its average signal intensity in order to account for plate-to-plate variability. Normalized values were used for all linear regression analyses.

**Statistical analyses**

All data was analysed using JMP Genomics software V5 (SAS Institute, Cary, NC.). Data was examined for normality, and linear regression was used to examine associations between FA and cytokines, and included sex, age, ethnicity and BMI as covariates because these variables are known to influence inflammation [20]. A Bonferroni correction was used to account for multiple testing.
3. Results

Study population characteristics

General characteristics of our study population are outlined in Table 6. Briefly, anthropometric and clinical measurements indicated that our population was in the normal range for all parameters. Further, low hsCRP levels (0.89 ± 0.04mg/L) reinforced that our study population showed no sign of inflammation.

<table>
<thead>
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<th>Mean ± SEM</th>
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<tr>
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<td>IFN-γ (pg/mL)</td>
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<td>IP-10 (pg/mL)</td>
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<tr>
<td>PDGF-ββ (pg/mL)</td>
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Table 6: TNH study population characteristics. Data are represented as mean ± SEM. BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein, TG, triglycerides; hsCRP, high sensitive C reactive protein, RANTES, regulated upon activation normal T-cell expressed and secreted, IL-1Ra, interleukin-1 receptor antagonist, IFN-γ, Interferon-γ, IP-10, interferon-γ inducible protein 10, PDGF-ββ, platelet-derived growth factor ββ.
Circulating FA levels

The mean concentrations of all FA detected in our population are presented in Table 7. FA contributing to <0.25% of the total FA profile are not listed because they could not be consistently detected in all study participants. The plasma FA profile was as expected, i.e. the most abundant FA were linoleic (2165 ± 19 µM), palmitic (1610 ± 17µM), and oleic acid (1272 ± 14 µM), while eicosapentanoic acid (37 ± 0.8 µM) and docosahexaenoic acid (89 ± 1.2 µM) were amongst the least abundant.

<table>
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<td>µg/mL ± SEM</td>
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<td>Stearic Acid</td>
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<td>Linoleic Acid</td>
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<td>2165 ± 19</td>
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<td>Docosahexaenoic Acid (DHA)</td>
<td>29.3 ± 0.4</td>
<td>89.4 ± 1.2</td>
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</table>

Table 7: Mean circulating concentration of FA in fasting plasma of the TNH study cohort. FA contributing to less 0.25% of the total FA profile are not included. Data are represented as mean ± SEM. Each fatty acid is expressed in µg/mL ± SEM and µM ± SEM.
**SFA and Markers of Inflammation**

Associations between individual SFA and markers of inflammation are outlined in Table 8. Myristic acid (14:0) was positively associated with hsCRP when expressed as both relative % (r=0.37, p<0.0001) and absolute (r=0.39, p<0.0001) values. Myristic acid was also significantly positively associated with the five cytokines studied when expressed as relative % and absolute values. Palmitic acid (16:0) was similarly associated with hsCRP when expressed as relative % (r=0.43, p<0.0001) and absolute (r=0.39, p<0.0001) values; however, palmitic acid was only positively associated with RANTES, PDGF-ββ, and IP-10, and not IL-1Ra or IFN-γ.

Stearic acid (18:0) was significantly inversely associated with hsCRP (r=0.39, p<0.0001) and RANTES (r=0.28, p<0.0001) when expressed as a relative %, but not absolute value. No additional associations were observed between stearic acid and the other four cytokines measured.

**MUFA and Markers of Inflammation**

Associations between individual MUFA and markers of inflammation are outlined in Table 9. When expressed as relative % and absolute values, palmitoleic acid (16:1n7) was positively associated with hsCRP (r=0.39, p<0.0001 and r=0.13, p<0.0001, respectively). Palmitoleic acid was also positively associated with the five cytokines when expressed as both relative % and absolute values.

Oleic acid (18:1n9) and cis-vaccenic acid (18:1n7) were positively associated with hsCRP when expressed as absolute concentrations (r=0.36, p<0.0001 and r=0.36, p<0.0001, respectively); however, these associations were not observed when oleic and vaccenic acids were expressed as a relative %. Furthermore, neither of these FA were associated with the five cytokines following adjustment for multiple testing.
**PUFA and Markers of Inflammation**

Associations between individual PUFA and markers of inflammation are outlined in detail in Table 10. Linoleic acid (18:2n6) was inversely associated with hsCRP ($r=-0.38$, $p<0.0001$) when expressed as relative %, as well as with RANTES ($r=-0.26$, $p<0.0001$) and PDGF-ββ ($r=-0.21$, $p<0.0001$). However, no significant associations were found when linoleic acid was expressed as an absolute value.

When dihomo-γ-linolenic (DGLA, 20:3n6) was expressed in both relative and absolute values, we observed a positive association with hsCRP ($r=0.42$, $p<0.0001$ and $r=0.44$, $p<0.0001$, respectively). DGLA was also significantly positively associated with the five cytokines. Arachidonic acid (20:4n6) was significantly positively associated with PDGF-ββ only when expressed as an absolute concentration ($r=0.16$, $p=0.0002$). No associations were observed when arachidonic acid was expressed as a relative %.

We did not detect significant associations between other common PUFA such as α-linolenic (18:3n3), EPA (20:5n3), DHA (22:6n3), and any inflammatory markers. When examining FA that were consistently detected, but in low abundance (i.e. γ-linolenic acid (18:3n6), eicosadienoic acid (20:2n6), adrenic acid (22:4n6) and DPAn-3 acid (22:5n3)), we found no evidence of relationships with inflammation (data not shown).
Table 8: Associations between SFA and inflammatory markers in the TNH study cohort. Linear regression analyses included sex, age, ethnicity, and BMI as covariates. Each FA is expressed as relative (%) or absolute (µg/mL) concentration. p<0.05 denotes a significance. *denotes significance after correcting for multiple comparisons.
Table 9: Associations between MUFA and inflammatory markers in the TNH study cohort. Linear regression analyses included sex, age, ethnicity, and BMI as covariates. Each FA is expressed as relative (%) or absolute (µg/mL) concentration. p<0.05 denotes a significance. *denotes significance after correcting for multiple comparisons.

<table>
<thead>
<tr>
<th></th>
<th>Palmitoleic acid (16:1n7)</th>
<th>Oleic acid (18:1n9)</th>
<th>Vaccenic acid (18:1n7)</th>
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<tr>
<td></td>
<td>Relative (%)</td>
<td>Absolute (µg/mL)</td>
<td>Relative (%)</td>
</tr>
<tr>
<td></td>
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<td>p-value</td>
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<td>hsCRP</td>
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</tr>
<tr>
<td>PDGF-ββ</td>
<td>0.212</td>
<td>&lt;0.0001*</td>
<td>0.212</td>
</tr>
</tbody>
</table>
Table 10: Associations between PUFA and inflammatory markers in the TNH study cohort. Linear regression analyses included sex, age, ethnicity, and BMI as covariates. Each FA is expressed as relative (%) or absolute (µg/mL) concentration. p<0.05 denotes a significance. *denotes significance after correcting for multiple comparisons.
4. Discussion

There is a growing appreciation that grouping FA together within a class (i.e. SFA, MUFA, or PUFA) presents an overly simplified scenario. Indeed, individual FA might have distinct roles compared to other FA within the same class. Due to the recognized links between FA and inflammation, the present study sought to examine the relationships between individual FA and a panel of inflammatory markers in a population of young adults. Using both a common clinical marker of inflammation (hsCRP) as well as a panel of cytokines provided a robust approach to study the associations between FA and inflammation. Many FA in the present study were strongly and positively associated with inflammatory markers; however, stearic and linoleic acids were inversely associated with these markers. Together, this study highlights the importance of considering individual FA as we seek to better understand how FA may influence inflammation.

While SFA have long been thought to promote inflammation [2], recent work examining individual SFA and their impact on inflammation has questioned this idea [21]. Some generalizations may stem from instances when the term “SFA” is used to describe results obtained from studies where only palmitate was used [22]. However, as shown in the present analysis, extrapolating results obtained with palmitic acid to other SFA is potentially erroneous. This is exemplified by our finding that stearic acid was inversely associated with hsCRP and RANTES. The lack of a positive association between stearate and inflammatory markers agrees with previous work by Fernandez-Real et al. [23]. This suggests that stearic acid has an anti-inflammatory or neutral effect on inflammation. In contrast, we report strong positive associations for both myristic and palmitic acids with markers of inflammation. This finding is strengthened by the fact that these associations were observed when expressing FA data as both
relative and absolute values. While it is currently unclear why stearic acid differs in its association with inflammatory markers compared to myristic and palmitic acids, one possible explanation may have to do with their differential regulation of toll-like receptors [21, 24]. Toll-like receptor 2 (TLR2) and 4 (TLR4) are cell-surface receptors that regulate an intracellular cascade of events that leads to the activation of NFκB and increased expression of pro-inflammatory cytokines such as IL-6 and TNFα [25]. Previous work by Lee and colleagues has shown that shorter-chain SFA such as lauric and palmitic acid can activate both TLR2 and TLR4 [25], however, the effect of longer-chain stearate was not explored. Recently, our group investigated the influence of individual FA on adipocyte gene expression and found that palmitate regulated the TLR signalling pathway, while stearate had no effect [24]. Together, this suggests that stearic acid may operate by another mechanism that leads to distinct effects compared to shorter-chain SFA. Thus, our results lend further support to the notion that not all SFA are pro-inflammatory [21].

MUFA have been reported to lower the risk of cardiovascular disease through anti-inflammatory effects [26]; however, current research also questions whether all MUFA have the same impact on inflammation [7]. The present study has found that palmitoleic acid, expressed as either relative % or absolute values, was positively associated with all inflammatory markers. Although recent evidence suggests palmitoleic acid has favourable effects on insulin action and lipid profiles [27, 28], the association with inflammation appears less positive. Zong et al. recently reported that palmitoleic acid was associated with an adverse adipokine profile [29]. This aligns with epidemiological data reporting that high levels of palmitoleic acid are associated with metabolic disorders [30, 31]. Our results agree with these previous studies, as we found that circulating palmitoleic acid levels are positively associated with a panel of inflammatory
markers. In contrast to palmitoleic acid, only weak associations were detected between oleic acid and inflammatory cytokines; however, after accounting for multiple testing all but one were statistically insignificant. This suggests that oleic acid has, at best, a negligible effect on inflammation. Our findings are consistent with several past studies that have shown oleic acid doesn’t seem to induce inflammation [1, 32, 33].

PUFA have garnered considerable interest in recent times. The general consensus is that n-3 PUFA are anti-inflammatory, while n-6 are considered pro-inflammatory [2]; however, it appears that the notion that n-6 PUFA are pro-inflammatory, in particular, warrants continued investigation [2-4, 34, 35]. EPA and DHA are well recognized to exert anti-inflammatory effects [2, 36]; however, we did not find any statistically significant associations with inflammatory markers in this study. This is likely due to the absence of overt signs of inflammation (i.e. population average hsCRP was < 1.0 mg/L) in our study population comprised of young healthy adults. Accordingly, it is hypothesized that n-3 PUFA display anti-inflammatory effects only when there is an inflammation (chronic or acute) present. This idea is supported by intervention studies in which n-3 PUFA supplementation did not change circulating CRP levels in healthy middle-aged adults [13, 37].

When considering n-6 PUFA, our current results add to the growing evidence that n-6 PUFA may not be pro-inflammatory themselves [3, 4]. Linoleic acid has received recent attention since a systematic review of human studies concluded that an association between linoleic acid and inflammation is not supported by current literature [35]. Building on results recently reported by our group in which we showed that linoleic acid was inversely associated with hsCRP [38], the current study extends these previous findings by showing that linoleic acid is also inversely associated with other circulating inflammatory markers (RANTES and PDGF-
ββ). Further, results from our group align with those of others [34], suggesting that linoleic acid is not pro-inflammatory and may, in fact, be anti-inflammatory.

It is noteworthy to mention dihomo-γ-linolenic (DGLA) was positively associated with all markers of inflammation. DGLA is derived from linoleic acid and is the immediate precursor for arachidonic acid. Both DGLA and arachidonic acid can be converted into eicosanoids, which are FA-derived metabolites known to regulate inflammation. DGLA is converted into anti-inflammatory eicosanoids, whereas arachidonic acid is converted into pro-inflammatory eicosanoids [36]. The strong positive associations found between DGLA and inflammatory markers seems counter-intuitive when considering that DGLA is used to produce anti-inflammatory eicosanoids; however, we hypothesize that this positive association may arise in order to maintain an equilibrium in inflammatory status. In other words, individuals may have higher levels of DGLA in order to offset higher baseline pro-inflammatory markers. Further investigation is required in order to substantiate this hypothesis.

Since the current cross-sectional study recruited only young healthy subjects, we were unable to robustly measure a number of the common cytokines typically used to study inflammation (e.g. TNF-α and IL-6) because they were below our detection threshold. Nevertheless, the cytokines reported in the current study provide intriguing clues regarding the extent to which FA can mediate inflammation. For example, RANTES is a pro-inflammatory chemokine that recruits leukocytes to sites of inflammation [16]. Elevated RANTES concentrations have been observed in several conditions comprising an inflammatory component, such as obesity [20]. Recent work by our group using 3T3-L1 adipocytes demonstrated that RANTES gene expression is regulated by SFA and MUFA [24]. Thus, FA regulation of RANTES may have important metabolic consequences and existing evidence
suggests this link should be further explored. Given the high-fat dietary habits in Western society, understanding the physiological and molecular implications of FA-mediated regulation of inflammation is paramount.

We acknowledge some limitations with the current study. Firstly, our population is comprised of young healthy adults; thus the associations observed here may not apply to older or diseased populations. Also, the cross-sectional nature of this work doesn’t allow us to draw any conclusions regarding cause and effect. This could only be elucidated in intervention studies aimed at unravelling the roles of specific FA on inflammation. However, one of the major strengths of this study is that we examined FA-inflammation associations using both FA expressed as both relative and absolute values. This approach increases the robustness of our conclusions and prevents reporting potentially false associations [39].

In conclusion, the current work highlights that not all FA from the same class are similarly associated with inflammatory markers. When considering the findings for SFA and MUFA together, our results may point to a structural basis for these associations, i.e. FA with ≤16 carbon chains are pro-inflammatory in contrast to FA with 18+ carbon chains. Further, our findings for stearic and linoleic acids suggest that further investigation of these two FA in particular is warranted. These results reinforce the relevance of examining the link between individual FA and inflammation as we attempt to better understand the contribution of FA to the development of chronic metabolic diseases.
5. Acknowledgements

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6. References


5. Voon PT, Ng TK, Lee VK, and Nesaretnam K (2011) Diets high in palmitic acid (16:0), lauric and myristic acids (12:0 + 14:0), or oleic acid (18:1) do not alter postprandial or fasting plasma homocysteine and inflammatory markers in healthy Malaysian adults. Am J Clin Nutr 94 : 1451-1457


38. Roke K, Ralston JC, Abdelmagid S, Nielsen DE, Badawi A, El-Sohemy A, Ma DW, and Mutch DM (2013) Variation in the FADS1/2 gene cluster alters plasma n-6 PUFA and is
weakly associated with hsCRP levels in healthy young adults. Prostaglandins Leukot Essent Fatty Acids