

Using Latent Class Analysis to Create Allostatic Load Profiles to Investigate the Effects of
Occupational and Perceived Stress in Firefighters

by
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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners. I understand that my thesis may be made electronically available to the public.

Abstract

Background: Allostatic load is a construct used to assess the sum of the effects of physiological stress across multiple body systems over time. Allostatic load is typically measured using the allostatic load index (ALI); however, this measure does not fully capture the multivariate nature of allostatic load. Using allostatic load profiles as opposed to the commonly used ALI, we hope to explore the multivariate nature of allostatic load and understand its association with perceived stress, since the existing literature exploring the association between perceived and physiological stress is inconclusive.

Objectives: The objectives of this thesis are to 1) develop allostatic load profiles using latent class analysis of both study-created and clinical-based thresholds for biomarkers of stress; 2) examine the association between allostatic load profiles and perceived stress in firefighters; 3) assess whether study-based or clinical-based thresholds are more suitable when measuring stress in the firefighters in question.

Methods: Using available biomarker data from a sample of 57 male firefighters in Waterloo Fire Rescue, we developed allostatic load profiles using latent class analysis. Biomarkers included systolic blood pressure (SBP), diastolic blood pressure (DBP), hemoglobin A1c (HbA1c), low-density lipoprotein (LDL), high-density lipoprotein (HDL), heart rate variability (HRV), cortisol, waist to hip ratio (WHR) and body mass index (BMI). We then employed logistic regression to assess the association between allostatic load profiles and perceived stress in these firefighters.

Results: Our results demonstrated that the use of allostatic load profiles (ALPs), created with study-based thresholds, showed how different biomarkers contribute to elevated or non-elevated physiological stress profiles. In regression models of ALP on Perceived Stress Scale (PSS-10) scores, we saw a consistent positive association such that an increase of 1 unit in PSS-10 score increased the odds of being in the elevated ALP group anywhere between 13.6% (OR = 1.136, 95% C.I. = 1.012, 1.299) in a model with PSS-10 only to 19.2% (OR = 1.192, 95% C.I. = 1.039, 1.400) in a model with PSS-10, length of service (LOS), and the following behavioural confounders: smoking, sleep hours, exercise and alcohol intake frequency.

Conclusions: Allostatic load profiles captured the multivariate nature of allostatic load and demonstrated a significant association with the PSS-10, whereas ALI was unable to significantly demonstrate a relationship with perceived stress. The results of this thesis also demonstrated the need for using study-based or study-specific thresholds when examining unique populations with different fitness levels than the general population. Further research can benefit from the use of allostatic load profiles in conjunction with study-based thresholds to accurately and completely address the needs of persons working in high-stress, dangerous occupations.

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Introduction

In the domain of occupational health, this thesis explored the important question of utilizing a multivariate measure/summary to quantify physiological stress in human subjects. This thesis also explored the association between physiological stress and perceived stress. Current literature on the topic of quantifying stress describes two broad approaches, namely measuring multiple biomarkers (physiological stress) and administering self-reported surveys like the Perceived Stress Scale (PSS-10) (perceived stress) (Dorsey et. al, 2022). *Physiological stress* can be assessed through the measurement of multiple biomarkers such as heart rate, blood pressure, obesity, etc., where each biomarker reflects a different aspect of physiological stress. In comparison, the use of self-reported surveys captures the notion of *perceived stress*. In the biomarker perspective, stress experienced by the human body is reflected in the multivariate construct known as allostatic load. Until now, researchers have used a single index score called the allostatic load index (ALI) to measure physiological stress in humans. However, the ALI is a summary score of overall stress and therefore does not capture the multifaceted nature of allostatic load.

This thesis proposed a multivariate approach to measuring allostatic load, which we called the Allostatic Load Profile (ALP), using the statistical technique of latent class analysis (LCA) and the same suggested set of biomarkers employed to measure ALI. This new methodology of deriving ALP is the first of its kind and the expectation is that it will be more accurate at assessing the multivariate construct of allostatic load due to its multivariate nature. Given that occupational stress is proposed to be associated with allostatic load, this thesis also

explored associations between ALP and occupational stress using an existing sample of firefighters from Waterloo Fire Rescue.

Occupational stress arises when an imbalance occurs between the demands of an individual's work and their physiological and psychosocial capacities to handle these demands (Lagrosen & Lagrosen, 2022). Occupational stress is worsened when workplaces do not provide adequate resources to help their employees manage and cope with occupational stress.

Occupational stress is a health concern for many individuals in modern society (Lagrosen & Lagrosen, 2022). Stressful and even toxic work environments are not uncommon across various career paths, from office jobs to skilled trades. However, certain career paths come with greater exposure to occupational stress than others due to the nature of the occupation. An example of such a group is first responders, which include emergency medical service (EMS) providers, law enforcement officers and firefighters. First responders, due to their exposure to life-threatening situations, are more prone to mental health and substance abuse issues compared to many other occupations (Doyle et al., 2021). Furthermore, due to the life threatening nature of the work of first responders, they should be given the tools to perform to the best of their abilities with complete mental clarity. Therefore, understanding how occupational stress impacts the health and well-being of first responders is important to study in occupational health research.

Firefighters, among first responders, face unique challenges that contribute to occupational stress. Firefighters are asked to put their bodies and lives on the line as they enter dangerous situations in which they are exposed to extreme levels of heat and harmful smoke, as well as the physical instability of the buildings they enter. Rajabi et al. (2020) found the most prominent operational stressors for firefighters include exposure to toxic fumes and fear of explosions. These operational stressors to physical health contribute to mental stress for

firefighters, which can have serious implications for remaining safe on the job. In fact, Smith et al. (2020) found that firefighters coping poorly with stress were more likely to experience burnout and pay less attention to safety precautions. Unresolved stress is particularly dangerous in firefighters due to the nature of their work. Exhaustion, poor cognition, or high allostatic load can have serious consequences for firefighters because they are constantly exposed to life-threatening situations.

Allostatic load is a construct to reflect the physiological impact of chronic stress on the body, and is typically assessed by examining various biological markers (Guidi et al., 2020). Commonly used biomarkers include, but are not limited to, cortisol levels, systolic blood pressure (SBP), diastolic blood pressure (DBP), and body mass index (BMI) (Beese, Postma & Graves, 2022). An elevated allostatic load is not only an indication of excess chronic stress, but is also associated with negative health outcomes (Rodriquez et al., 2019). For example, high allostatic load is associated with immunosuppression, reduced insulin sensitivity and other precursors to metabolic disorders (Kinlein, Wilson & Karatsoreos, 2015). Quantifying allostatic load in the current literature is done through an ALI, which is a value constructed using various biomarkers. The details of computing ALI are discussed in Section 4.2.

Since the focus of this thesis is on the development of a new multivariate summary of allostatic load, and the assessment of its association with occupational and perceived stress, we heavily referenced the earlier work of Igboanugo et al. (2022), in which (i) ALI was used to capture overall stress and (ii) general life stress was assessed using the PSS-10 (see Appendix A). Igboanugo et al. (2022) found the association between ALI and occupational stress to be non-significant. This finding may have been influenced by the major limitation of ALI, namely that it is a simple score for a multi-dimensional construct. The proposed shortcoming of ALI is

our motivation for proposing *profiles* for allostatic load, as opposed to an *index*. Through ALP, this thesis will capture the multivariate nature of elevated or non-elevated allostatic loads among firefighters from Waterloo Fire Rescue. This thesis will also investigate the perceived stress-related determinants of ALP. Understanding the multivariate nature of allostatic load and its association with occupational stressors can help inform multipronged, targeted interventions to improve the quality of life and health outcomes for first responders such as firefighters.

Chapter 1. Background & Literature Review

1.1 Background

Occupational stress can impact anyone regardless of their occupation. Whether it be a 9-5 office job, a night shift on a production line, or unpredictable on-call hours for emergency physicians. Occupational stress may occur when individuals do not have the capacities required to meet the demands of their occupation, whether these capacities be emotional, financial, physical, or social. When occupational demands increase, occupational stress can be exacerbated without increased resources to employees.

Occupational stress poses health risks to employees when the stress is persistent and beyond their ability to cope (Lagrosen & Lagrosen, 2022). While occupational stress impacts employees of all occupations, certain occupations come with additional stressors. Many occupations, such as corporate jobs, expose employees to predominantly psychosocial stressors such as difficult co-workers or management, or high workloads. However, certain occupations also come with physical stressors. One such occupation is firefighting. In addition to common psychosocial stressors, firefighters are also exposed to a host of physical stressors such as exposure to high temperatures, inhalation of toxic fumes, and carrying heavy equipment (Rajabi et al. 2020). In an occupation with as many potential dangers as firefighting, it is important to address occupational stress. High levels of occupational stress can impact performance in any occupation; however, this is particularly dangerous for firefighters, given the physical demands and dangers inherent in their occupation.

1.2 Biomarkers of Allostatic Load

Allostatic load was first developed by McEwen and Stellar in 1993 to quantify chronic stress (Servant-Fine et. al, 2023). In the mid-2000s, allostatic load became a more commonly used tool

for quantifying chronic stress. Allostatic load was used to evaluate the “wear and tear” that chronic stress causes on the human body. Allostatic load is determined using a collection of biomarkers, with the most commonly used mix of biomarkers being body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), high-density lipoprotein (HDL), low-density lipoprotein (LDL), Hemoglobin A1c (HbA1c), heart rate variability (HRV), waist to hip ratio (WHR) and cortisol (Rodriguez et al., 2019). Cortisol is the most consistently used biomarker to evaluate allostatic load, although it cannot be used alone to evaluate allostatic load (Beese, Postma & Graves, 2022). In studies focusing primarily on the metabolic impacts of chronic stress, HDL, cholesterol, HbA1c, BMI, and WHR were the most frequent biomarkers used to compute ALI (Beese, Postma & Graves, 2022).

While the aforementioned biomarkers are not an exhaustive list of all biomarkers that can be used to measure allostatic load, a lack of consensus exists on the precise set of factors that should be used in this regard. While the initial study by McEwen and Stellar focused on 10 biomarkers, and these are the most commonly used biomarkers to assess allostatic load, different studies use varying combinations, largely due to practical issues such as cost and viability (Servant-Fine et. al, 2023). To take a case in point, recent studies have suggested that WHR is a better health measure than BMI because the latter fails to account for muscle mass (Cheng et al., 2010). Furthermore, WHR is a better predictor of abdominal obesity, which has been found to be more detrimental to health than obesity in other regions of the body such as the arms and legs (Cheng et al., 2010). WHR is also an optimal obesity marker for men because they are more likely to store fat in their midsection (Nauli & Matin, 2019).

The biomarkers described above can be labeled as either primary or secondary mediators, with primary mediators being cortisol, epinephrine, and other hormones produced when the HPA

axis is activated in response to stress (Beese, Postma & Graves, 2022). The secondary mediators, including blood pressure and waist to hip ratio, are the physical changes resulting from dysregulation of the primary mediators (Beese, Postma & Graves, 2022).

1.3 Occupational Stress in Firefighters

Occupational or workplace stress is a large contributor to ill-health. Some employers may ask employees to take on unreasonable workloads (Lagrosen & Lagrosen, 2022). Certain careers also come with more exposure to occupational stress than others. First responders such as firefighters, emergency medical service providers and law-enforcement officers generally experience more occupational stress than persons in other professions (Doyle et al., 2021). While occupational stress in many jobs frequently stems from high workloads, a negative company culture or lack of autonomy (Lagrosen & Lagrosen, 2022), occupational stress for first responders can also come from exposure to life-threatening situations and traumatic events. For example, first responders, may be called to motor vehicle accidents, natural disasters, or serious medical emergencies (Kimbrel et al., 2015). First responders may also be under more stress while performing their work as the consequences of making a mistake are far greater than in many other occupations. Firefighters, in particular, experience the stress of entering potentially life-threatening environments where they face the risk of severe burns, explosions, smoke inhalation and toxic fume inhalation. Furthermore, firefighters also deal with traumatic scenes (e.g., gruesomely disfigured corpses) in addition to common workplace stressors such as poor management and work culture. Workplace stress can become chronic stress, which can lead to a persistent feeling of being overwhelmed. Furthermore, chronic stress can lead to alterations of the HPA axis and stress response, as previously mentioned.

Chronic stress can negatively impact health in many ways, such as leading to chronic elevated blood pressure, suppressed immune function, and insulin resistance, among other issues (McEwen, 2005). These health conditions can contribute to the development of metabolic diseases such as cardiovascular disease and type 2 diabetes. Going beyond the individual, chronic diseases place a large burden on health and society because they are the most common diseases in the modern world, taking many lives and costing health care systems a great deal of money each year. In fact, in 2018, the cost of chronic diseases to the health care system in Ontario was estimated to be \$10.5 billion (Cancer Care Ontario, 2019). According to a 2022 study by Kim et. al, male firefighters are at an increased risk of myocardial infarction (MI) due to stress, among other factors (standardized incidence ratio [SIR] = 1.16, compared to males working in other occupations), as well as an increased risk of angina and chronic ischemic heart disease. This increased risk of MI is in part due to stress from numerous factors in the occupation of firefighting, particularly abnormal sleep hours. Developing a more accurate measure of physiological stress using ALP could be more useful in future studies that explore the impact of physiological stress on health outcomes. A measure such as ALP will produce a higher quality of evidence to inform the selection of better targeted interventions to mitigate the risks of chronic diseases associated with prolonged physiological stress.

1.4 Occupational Stress Scales

As occupational stress has become an increasingly important topic when discussing health and chronic disease, several tools have been created to measure the construct. Tools that evaluate perceived and occupational stress, and the moderation of stress by social support, respectively, include the Perceived Stress Scale (PSS-10; Cohen, Kamarck, & Mermelstein, 1983) , the Sources of Occupational Stress Scale (SOOS-14; Kimbrel et al., 2011), and the

Multidimensional Scale of Perceived Social Support (MS-PSS; Zimet, et. al, 1990), and the Firefighter Social Support Scale (SSS-FF; Isaac & Buchanan, 2021). These scales are being discussed here to provide the audience with a brief introduction to the purpose of the scales as they will be used in the logistic regression models later in the thesis.

The PSS--10 is a tool used to measure perceived stress and it consists of 10 questions scored from 0 to 4, with 0 being never experiencing the feelings asked in the question and 4 being very frequently experiencing those feelings (Cohen, Kamarck, & Mermelstein, 1983). These questions focus on feelings of being upset over unexpected happenings in the most recent month. The responses to the 10 questions are aggregated to obtain a score from 0 to 40, with scores ≤ 13 indicating low stress and > 13 indicting high stress. The test-retest reliability of the PSS has been found to be above 0.70 and participant responses to the mental and emotional sets of questions within the scale were found to be correlated with one another (Lee, 2012).

Another relevant instrument is the Sources of Occupational Stress Scale, a 14-item inventory (SOOS-14) formulated to examine the impact of occupational stress on firefighters and other first responders (Kimbrel et al., 2011). This scale contains 14 domains that have been shown to be effective at measuring occupational stress in first responders, especially firefighters (Kimbrel et al., 2011). The scores for SOOS-14 can range from 14 to 70, with higher scores indicating greater levels of occupational stress. Furthermore, the range for internal consistency using the SOOS-14 is 0.78 to 0.84, indicating that it is a reliable measurement of occupational stress.

In addition to perceived stress scales, social support scales were also examined in terms of their association with ALP. The MS-PSS is a scale used to assess the extent to which an

individual feels supported, whereas the SSS-FF assesses specifically firefighters' perception of support from peers in the workplace.

1.5 Confounding Variables

Several confounders can affect the association between perceived stress and allostatic load. For our study, the list of confounders includes age, length of service (LOS), and behavioural variables consisting of smoking, sleep hours, exercise, and alcohol intake frequency. We regard age as a confounder because, as one ages, the body becomes less efficient at dealing with stress and the biomarkers used to compute ALI can be elevated in older firefighters. For example, with age, waist to hip ratio typically increases as muscle is lost and replaced with fat. This happens especially in males, who tend to carry more fat around their midsection compared to females (Nauli & Matin, 2019). Additionally, age is a risk factor for many diseases associated with stress, such as cardiovascular disease (CVD), diabetes, hypertension, and others. In contrast, LOS may appear protective against the effects of stress. The more years a firefighter has worked in the field, the more they are likely to be able to cope with stressors that come with the job. They may feel less nervous or have better coping mechanisms than newer firefighters, which may protect them both physiologically and psychologically. On the other hand, a longer LOS means an increase in age, which typically elevates allostatic load biomarkers. Furthermore, longer LOS means potentially increased responsibility, which may add to stress. It may also lead to the effects of stressful experiences being compounded over time (Makara-Studzińska, Wajda, Lizińczyk, 2020).

Furthermore, we also explored a set of behavioural variables as confounders. These variables were collected in the original study conducted by Igboanugo et al. (2022), include smoking, exercise, sleep hours and alcohol intake frequency. Behaviors such as smoking and

drinking in excess are known to be detrimental to health and they can influence allostatic load by elevating multiple biomarkers. Not only do these behaviours affect allostatic load, but they can also affect perceived stress as these are common coping mechanisms to combat stress and can cloud the association between perceived stress and allostatic load (McEwen, 2000).

Additionally, insufficient exercise is typically associated with having a higher allostatic load (Rodriquez et al., 2019), and this lack of exercise could be due to the perception of stress in the individual (McEwen, 2000). Another variable that can affect allostatic load, the perception of stress, and negative health outcomes in firefighters is sleep (McEwen, 2000, Kim et. al, 2022). Lack of sleep is associated both with an increased allostatic load and an increased perception of stress, which can affect how individuals report stress. In summary, the aforementioned behavioural variables can play an important role when assessing the association between perceived stress and allostatic load, and hence should be accounted for in the modeling of the association between perceived stress and allostatic load.

Chapter 2. Study Rationale

The allostatic load index (ALI) is a commonly utilized metric for assessing the effects of occupational stress on physiological functions related to long-term health (Rodriquez et al., 2019). As noted earlier, the ALI is limited in its ability to capture allostatic load because it is operationalized as a single score value for the effect of stress. Hence, ALI cannot reflect the multidimensional nature of allostatic load. Given this limitation of ALI, we developed a new measure called *allostatic load profiles* using Latent Class Analysis (LCA; Weller et al., 2020), which allows for the discovery of latent/hidden groups based on shared characteristics (Naldi & Cazzaniga, 2020).

Allostatic load profiles will allow researchers to observe the underlying differences between what constitutes “high”, “elevated”, or “altered” allostatic loads as opposed to “low”, “normal”, or “usual” allostatic loads. For example, in our application with data collected from a sample of firefighters, a high allostatic load profile could be due to elevated blood pressure and obesity, while a (relatively) low allostatic load profile could be purely due to elevated obesity. Such profiles can help differentiate the allostatic loads experienced by individuals and even populations, which could then inform interventions to improve population health. For example, if elevated SBP were found to contribute to increasing the allostatic load profile in firefighters, then we could apply targeted interventions such as stress management to reduce SBP in firefighters. Our methodological contribution of developing latent class profiles in the context of allostatic load can be adopted in other applications/contexts that utilize similar constructs.

Chapter 3. Objectives

Objective 1a.

We developed allostatic load profiles using LCA for our study sample of firefighters, based on SBP, DBP, HbA1c, LDL, HDL, HRV, cortisol, WHR and BMI, using clinically established thresholds. Since BMI and WHR are biomarkers for obesity, we developed allostatic load profiles separately using these biomarkers. Details about the biomarkers will be discussed in section 3.2.

Objective 1b.

We developed allostatic load profiles using study-based thresholds derived from biomarkers in our study sample. This method of creating thresholds were initially proposed by Seeman et. al. (1997) and used by Igboanugo et al. (2022). Similar to objective 1a, we developed allostatic load profiles separately using BMI and WHR as the obesity biomarker. Details about the biomarkers will be discussed in section 3.2.

Objective 1c.

We compared the allostatic load profiles from objective 1a and objective 1b using visual tools (radar plots) to assess the influence of thresholds (clinical versus study-based) on developed allostatic load profiles. The optimal model for ALP will be determined using model fit statistics, which are discussed in section 4.2.

Rationale for Objective 1:

Objective 1 aimed to develop latent class profiles that will be the basis to assess allostatic load in firefighters. The process of creating allostatic load profiles involved dichotomization of the biomarkers based on thresholds for determining elevated biomarker levels. Multiple ways exist to perform this dichotomization, for example, using clinical thresholds versus study-based thresholds. Therefore, we investigated whether allostatic load profiles are influenced by the dichotomization method. Since the clinical thresholds are a well-accepted standard in medical practice, and these standards were derived from individuals who may not be in the same physical health as firefighters, we also examined the validity of applying clinical thresholds to the firefighter's data. Comparing findings from both the study-based and clinical thresholds will help elucidate whether firefighters differ from the general population.

Objective 2a: We explored associations between perceived and occupational stress with Allostatic Load Profiles developed in objective 1a.

Objective 2b: We explored associations between perceived and occupational stress with Allostatic Load Profiles developed in objective 1b.

Objective 2c: We compared differences in the association between perceived and occupational stress and Allostatic Load Profiles from objective 2a and 2b, to gauge sensitivity of results when using clinical versus study-based thresholds for biomarkers.

Rationale for objective 2:

In Objective 2, this thesis examined whether the association between allostatic load profiles and occupational stress is influenced by the choice of clinical or study-based thresholds to dichotomize the biomarkers. Because the thresholds used to dichotomize biomarkers may influence ALP, it is important to investigate whether the choice of thresholds also influences the association between ALP and perceived and occupational stress.

Chapter 4. Study Methods

The City of Waterloo's Fire Rescue department employed 120 full-time firefighters at the time of Igboanugo et al.'s (2022) study. Sixty-four firefighters participated in Igboanugo et al.'s study and the thesis drew upon this sample as the source of data for the analyses described below.

Igboanugo et al. (2022) excluded firefighters undergoing medical treatment and those with pre-existing mental health conditions. The firefighters in the study completed several surveys to assess perceived and occupational stress, including the SOOS-14, PSS-10, MS-PSS, and SSS-FF described in subsection 1.4 above. Information on the following biomarkers was available from these participants: SBP, DBP, HbA1c, LDL, HDL, HRV, cortisol, WHR and BMI. These biomarkers were collected using well-informed methods: OMRON 3 Series blood pressure monitor was used for measuring SBP and DBP, A1CNow+ analyser was used to measure HbA1c, CardioChek 181PA analyser was used to measure HDL and LDL, Bittium Faros ECG Ambulatory Sensor recorded HRV, and cortisol was measured through laboratory analysis of hair samples. A detailed description of the process of data collection and these methods is provided in Igboanugo et al. (2022; section 7.3.5, pp. 180-182).

Igboanugo et al. (2022), using the standards suggested by Seeman et al. (1997), computed ALI by (i) dichotomizing each biomarker (e.g., cortisol, systolic blood pressure, diastolic blood pressure, etc.) as either a 0 or 1 (1 indicates the biomarker exceeded the threshold value), (ii) then computed the sum of the dichotomized biomarkers. For illustration, suppose a study has only 3 biomarkers: systolic blood pressure (SBP), diastolic blood pressure (DBP) and cortisol. In this illustration, also suppose one subject has high SBP (denoted as 1), high DBP (denoted as 1), and normal cortisol (denoted as 0), while a second subject has normal SBP (denoted as 0), high DBP (denoted as 1), and high cortisol (denoted as 1). For this example, both subjects will have

the same ALI score of 2 and would hence be (imprecisely) labelled as being similarly affected by stress. The ALI method of assessing allostatic load assumes that each biomarker contributes equally to allostatic load, which is an assumption that should not be made.

Regarding consideration of confounders, the original study by Igboanugo et al. (2022) adjusted for sex, age, LOS, smoking, alcohol, and hours of sleep. In their investigation about possible confounding effects of these variables, the authors found that the only statistically significant variable was age ($p\text{-value} = 0.04$). While the $p\text{-value}$ for LOS was low ($p\text{-value} = 0.06$), it was not statistically significant. For further details pertaining to the original study design, data collection methods, recruitment, and findings, we refer the readers to Igboanugo et al. (2022).

4.1 Data Inclusion/Exclusion Criteria

The 64 participants in Igboanugo et al.'s (2022) study included 6 female and 58 male subjects. The small number of female subjects is not sufficient to support the development of the models discussed in objectives 1 and 2 from Chapter 3. For this reason, female subjects were excluded from this thesis. Among the 58 male subjects, 5 participants with missing data on cortisol were included in our analyses since latent class analysis can handle such missingness. Lastly, 1 participant who was missing data on all variables and was excluded from our study. This led to a final sample size of 57 for the thesis.

4.2 Methods for Research Objectives

For this thesis, it was first important to perform power and sample size calculations for latent class models to inform the decision around the number of classes/profiles that could be pursued when developing the allostatic load profiles. In addition to the number of classes, the power and

sample size calculation also required specifying the level of distinctness between classes, which is a measure of the amount of dissimilarity between the developed classes ¹. Based on the work done by Dziak, Lanza & Tan (2014) and given our study data dimensions – sample size $N = 57$, number of items (biomarkers) $J = 8$ – we calculated that the level of distinctness that could be pursued for our study was $w = 0.63$ with a power (θ) of at least 70%. Using $(N, J, w, \theta) = (57, 8, 0.63, 0.70)$, the latent class analyses could only afford a two-class model, which was utilized to address objectives 1a and 1b. Additionally, we ran two sets of models, one with BMI as the obesity biomarker, and one with WHR as the obesity biomarker.

For objectives 1a and 1b, LCA was performed in three steps. Step 1: dichotomize each of the 9 biomarkers as “high-” or “low-” risk. Step 2: build the model using the dichotomized versions of the biomarkers. Step 3: visualize and interpret the developed classes. For Step 1 of LCA, we considered two ways of dichotomizing the 9 biomarkers – one using clinically established thresholds (for “elevated”) for each of the biomarkers, and the second using study-based thresholds (for “elevated”). For clinical thresholds, we referred to clinical guidelines, where possible, for delineating what is considered high-risk for each of the biomarkers. Clinical thresholds for the biomarkers listed in Table 1 below were employed by Bizik et al. (2013) to calculate ALI. Finally, for objective 1c, the “best” model for ALP was determined using model fit statistics Bayesian Information Criterion (BIC) and entropy², along with model interpretability.

¹ Distinctness between classes refers to the extent of overlap between the latent classes for each variable. In Dziak, Lanza & Tan (2014), the level of distinctness is denoted by the letter w with larger values of w suggesting that the developed classes are more distinct than similar.

² Entropy is an index designed to measure the degree of latent class separation, with higher values indicating better class separation.

Table 1: *Clinical Thresholds for Biomarkers Used in this Thesis*

Biomarker	Clinical Normal Range	Coding for “elevated risk”
SBP	90-140 mmHg	≥ 140 mmHg is coded as 1, 0 otherwise
DBP	60 to 90 mmHg	≥ 90 mmHg is coded as 1, 0 otherwise
WHR	0.8 to 1	≥ 1 is coded as 1, 0 otherwise
BMI	18.5 to 25 kg/m ²	≥ 25 kg/m ² is coded as 1, 0 otherwise
HDL	0.9 to 2.0 mmol/L	≤ 0.9 mmol/L is coded as 1, 0 otherwise
HbA1c	4.6 to 6.2 mmol/L	≥ 6.2 mmol/L is coded as 1, 0 otherwise

Note: Clinical Normal Range values come from Bizik et al. (2013; **Figure 4, p. 306**).

Regarding clinical thresholds for biomarkers, Table 1 does not include cut-off values for LDL, HRV or cortisol because these biomarkers do not have clearly established or accepted clinical cut-off values across existing literature. Various issues exist with the clinical cut-off values for each of the variables. Let us address each of these three biomarkers, starting with cortisol. This thesis used data from Igboanugo et. al, (2022) which included HCC as the cortisol measure; this method, while non-invasive and stable, still does not have a set or typical measure seen in adults. For example, a recent systematic review by Igboanugo et al. (2023) attempted to confirm a clinical cut-off point for HCC, but they were unable to determine a firm cut-off value due to variance between different vendors of test kits. Regarding LDL, accurate means of measuring this biomarker are expensive and rarely used in studies. More commonly, the LDL data from the original study by Igboanugo et. al, (2022) was determined through a simple lipid blood panel, and this is typically an inaccurate estimate (Johns Hopkins Medicine, 2021). When looking at HRV, it is important to note that what constitutes a normal or abnormal HRV is highly variable

with variability dependent on several factors such as age, gender, and environment in which HRV is measured (lab setting versus in home) (Milicević, 2001). Igboanugo et. al. (2022) assessed HRV for only 5 minutes, which may not be long enough to accurately assess HRV. To address this hurdle, we used study-based thresholds for these biomarkers. This approach is a limitation to the goal of objective 1a because it is technically a hybrid as opposed to a purely clinical-based means of calculating biomarker thresholds.

Turning to study-based thresholds, these were devised by obtaining the 25th and 75th percentiles of biomarkers in our study sample dataset. The 75th percentile was used to indicate elevated values for SBP, DBP, WHR, BMI, LDL, HbA1c, and cortisol, whereas the 25th percentile was used for HDL and HRV (Seeman et al. ,1997; Igboanugo et al., 2022). We used different percentile cut-off values because some biomarkers, e.g., BMI, more adversely affect allostatic load when they exceed the normal range of values. However, biomarkers like HDL pose more risk to the body when they are low.

For Step 2 of LCA, we developed the latent class models using the poLCA package (Linzer & Lewis, 2011) in R version 4.2.2 (The R Foundation for Statistical Computing, Vienna, Austria), with the number of classes set to 2 and using the dichotomized version of the 9 biomarkers. For Step 3 of LCA, we visualized the fitted classes in radar plots based on the work of Adams, Jimi & Lippert (2019), and in these plots we incorporated several recommendations from Dr. Ashok Chaurasia to improve model interpretation (Dr. Ashok Chaurasia, personal communication, August 2023). These recommendations will be discussed as we report our findings from LCA in Section 5.2.1.

For objective 2, we built logistic regression models using R's glm2 package (Marschner, 2011) to assess the association between allostatic load profiles (generated via LCA) and

perceived stress. Separate models were built using ALP based on clinical thresholds (and study-based thresholds for LDL, HRV or cortisol) and another using ALP based entirely on study-based thresholds. These models regressed ALP (hybrid- or study- based) on PSS-10 (as described in section 1.4), while controlling for the effects of age or LOS. Age or LOS, as well as behavioural confounders, were included as control variables in separate models to mitigate their confounding effects. Specifically, the key models built included (i) ALP regressed on PSS-10 alone, (ii) ALP regressed on PSS-10 and adjusting for LOS, and lastly, (iii) ALP regressed on PSS-10 and adjusting for LOS and behavioural confounders. Finally, we compared results from these three logistic regression models by looking at the significance, effect sizes, model fit statistics, and model interpretation. These results were also compared to the findings from Igboanugo et al. (2022) to highlight differences between ALI and ALP.

The two model-fit statistics utilized in this thesis were the Bayesian Information Criterion (BIC) and entropy. BIC is commonly used to reflect the notion of parsimony, which is a balance between model fit and complexity, and is arguably the most common criterion for model selection. There is no cut-off point for BIC and its results are measured relative to the BIC values for other competitive models of interest, where preference is given to the model with lower BIC. Entropy, like BIC, is reported and used as a model selection guide and the interpretation of its absolute numerical value is unimportant for model selection. Rather, we look at the entropy values across models and the higher value is preferred. On the note of a cut-off of values for entropy, there is no universally accepted value, and nor should there be such an accepted value, since each study is unique with their own sample size, and model(s) of interest, and entropy is function of these two entities. Weller et al (2020) suggested that publishing LCA results gets

difficult when not including entropy values, and therefore suggested its reporting while emphasizing that entropy not be used as the sole metric for model selection in LCA.

Lastly, since this thesis involved the analysis of existing data, which was received in anonymized form, this thesis did not require ethics approval.

Chapter 5: Results

5.1 Power and Sample Size Calculations

5.1.1 Latent Class Analysis

Prior to running any models for objectives 1a and 1b, power and sample size calculations were performed using results from Dziak, Lanza & Tan (2014). The related formula is given by $N =$

$\frac{m_{\theta}^{(w^2)}}{w^2}$, where N represents the optimal sample size, w represents the degree of distinctness

between classes³, and $m_{\theta}^{(w^2)}$ represents a w -dependent constant for a target power of θ . To execute this formula, we first examined several graphs in Dziak, Lanza & Tan (2014, see pp. 544-545) of interest to extrapolate values for $m_{\theta}^{(w^2)}$ for different θ values. For illustrative purposes, a step-by-step breakdown of how this equation was applied in this thesis is given below:

Step 1. We chose an appropriate reference graph based on the number of biomarkers, which for our study was Figure 3 of Dziak, Lanza & Tan (2014, see pp. 544), since the number of biomarkers was $J = 8$.

Step 2. In the chosen graph, for a power value of θ on the y-axis, we got a corresponding value on the x-axis based on the power curve. For example, if the power value was $\theta = 70\%$, then the corresponding x-value for the curve was $m_{\theta}^{(w^2)} = N \times w^2 = 22$.

Step 3. Given our sample size ($N = 57$), we solved for w from step 2 to get $w = 0.63$.

In the above steps, if a power value of $\theta = 80\%$ was chosen, one would arrive at $w = 0.46$ for our sample size ($N = 57$). Similarly, if one wanted values for w greater than 0.63, then

³ The value of w can range between 0 and 1 with greater values closer to 1 being more ideal because they represent less overlap between the latent classes.

this would come at the cost of reduced power for our sample size of 57, i.e., $\theta < 70\%$. Given that higher values of w are preferred, we could only achieve a power of 70% with a distinctness of 0.63. The R-code for the aforementioned steps is shown in **Box 1** with calculations for different values for w , J and θ , which is followed by relevant results in **Box 2**.

```
#creating the function for equation 7, p.541 of Dziak, Lanza &
Tan (2014)
Lanza.2014.N=function(m,w) {
  N= m/(w^2)
  return(ceiling(N))
}

#grid search for m_power and N using inputs of J, power, and w
dat=expand.grid(J=c(7,8,9,10),
               power=c(0.7,0.8),
               w=c(0.25,0.5,0.63,0.75))

dat$mpower=c(18,22,30,35.5,21,26.4,27.2,30.8)
#these values come from Figure 3 for plot with J=8

dat$N=Lanza.2014.N(m=dat$mpower,w=dat$w)
```

Box 1: Function creation and grid search for sample size and power calculation for latent class models in Objective 1.

J	power	w	mpower	N
8	0.7	0.63	22.0	56
8	0.8	0.63	26.4	67

Box 2: Results for sample size and power calculation for latent class models in Objective 1.

With these results in **Box 2**, the power was estimated to be least 70%, with a distinctness value of 0.63. A value of 0.63 suggests the distinctness between classes is not exceedingly strong. Distinctness can be envisioned as the extent of non-overlap between two sets of a Venn diagram, where the two sets represent two latent classes. Hence, a value of $w = 0.63$ for our study means that the latent classes would more dissimilar than similar for our given sample size of $N = 57$.

5.1.2 Expected Power for Logistic Regression

To determine the power expected from our proposed logistic regression models in Objective 2, this thesis referred to power and sample size calculations by Chaurasia & Harel, 2013 (see equation 14, pp. 2774-2775) and personal communication with Dr. Ashok Chaurasia (personal communication, June 2024). The pertinent equation is given by

$$\phi_n(p, p_*) = \max \left\{ 0, 1 - \frac{0.5 (p - p_*)}{(n - p - 5)^{0.6}} \right\},$$

where $\phi_n(p, p_*)$ denotes the expected power (on average) at sample size n for a model with p_* number of predictor variables relative to the full model with p number of predictor variables. For the purpose of calculating power, we have given the R-function below:

```
power.func<-function(n,p,pstar) {  
  value= (1-0.5*(p-pstar)/(n-p-5)^0.6)  
  return(value)  
}
```

Box 3: Function for expected power for logistic regression models in Objective 2.

For a model where we suspect PSS-10 is associated with ALP, while the model already contains LOS, we calculated power of at least 95.1% by using the R-function as follows:

`power.func(n=57,p=3,pstar=2)`. Based on existing literature on allostatic load and models considered in Igboanugo et al. (2022; see study 4, pp. 319-320), we explored power calculations for models that account for confounders. Similar to Igboanugo et al. (2022), we considered 4 additional confounders from the behavioural domain: smoking (binary), sleep (ordinal with 3 levels), exercise (binary), and alcohol intake frequency (ordinal with 6 levels). This model reflects $p=11$ (9 indicator variables from the behavioural domain, LOS and one of the perceived stress variables: PSS-10, SOOS, MS-PSS or SSS-FF) and $p_*=10$ (9 indicator

variables from the behavioural domain and LOS), which results in a power of at least 94.6% by using the R-function as follows: `power.func(n=57, p=11, pstar=10)`.

5.2 Developing Allostatic Load Profiles (ALP) using Latent Class Analysis (LCA)

5.2.1 ALP using Study-based Thresholds for Biomarkers: WHR versus BMI (Objective 1a)

Using the dichotomized biomarkers based on study-based thresholds and WHR as the obesity biomarker, we developed allostatic load profiles using poLCA function in the R-package with the same name. These results are shown next.

Table 2: LCA results for ALP using study-based thresholds when WHR is used as the obesity biomarker.

Label	Class Size	Proportions by Biomarkers							
		SBPq75	DBPq75	WHRq75	HbA1cq75	LDLq75	cortisolq75	HRVq25	HDLq25
LC1	0.544	0.124	0.087	0.116	0.391	0.166	0.412	0	0.188
LC2	0.456	0.367	0.424	0.494	0.352	0.335	0.118	0.458	0.319
Prevalence	—	0.263	0.281	0.333	0.368	0.263	0.25	0.263	0.263

Note: “q75” reflects the corresponding variable’s 75th quartile, and similarly “q25” reflects the corresponding variable’s 25th quartile. This model had fit statistics as follows: BIC = 593.492, entropy = 4.632.

LC = Latent Class.

Based on the results from **Table 2**, it is estimated that 54.4% of the study population belonged to latent class 1 (LC1) while 45.6% of the study population belonged to latent class 2 (LC2). **Table 2** also shows that LC2 members were most likely to have elevated DBP and WHR compared to other biomarkers. The BIC for this model was 593.492 and the entropy was 4.632. To better understand the fitted profiles LC1 and LC2 from **Table 2**, we visualized these results in radar plots shown in **Figure 1** and **Figure 2** based on the work of Adams, Jimi & Lippert (2019), and these plots incorporated recommendations based on personal communications with Dr. Ashok Chaurasia (personal communication, August 2023) to facilitate communication of findings.

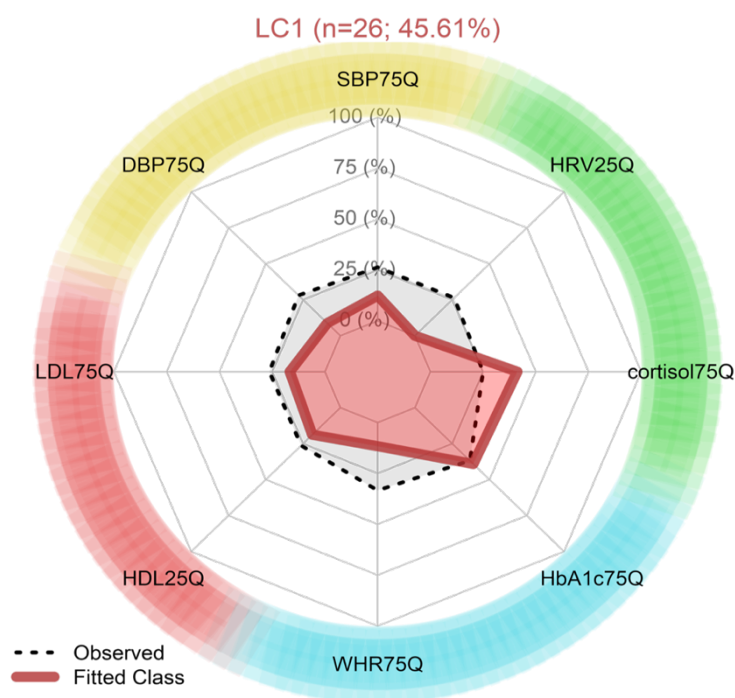


Figure 1: Visualizing results for LC1 from **Table 2:** LCA results for ALP using study-based thresholds when WHR is used as the obesity biomarker.

There are several areas to observe in **Figure 1**. First, we can see the 8 biomarkers displayed, each as a separate spoke (like spokes of a wheel) of the radar plot. These spokes are placed equidistant/equiangular from each other in a radial pattern. Tick marks on each spoke moving radially outward represent increasing proportions for elevated levels in the respective biomarkers. It can also be seen that certain biomarkers are grouped by colour to represent grouping, for example, SBP and DBP are grouped together under a yellow band because they are both blood pressure measurements, this grouping is one of the recommendations from Dr. Chaurasia. Another recommendation from Dr. Chaurasia is the inclusion of a grey shaded polygon outlined with a dashed line to represent the observed profile. This observed profile is determined from the proportion of cases in the sample that had elevated levels in the respective

biomarkers. The observed profile highlights the physical fitness of the sample of firefighters since the proportion values are approximately 25-30%. The LC1 profile, outlined in red, is mostly contained within the observed profile, with the exception being cortisol with a proportion at approximately at 40%. Hence, we classified/labelled LC1 as “normal” or non-elevated. The number of subjects categorized into LC1 was 26 of the 57, which constitutes 45.6% of the firefighters from the study sample. The inclusion of class size (count and percentage) in the title for the radar plots is another recommendation from Dr. Chaurasia. Hence, with our two-class model, ALP was dichotomized as class LC1 or LC2, with LC1 serving as the reference category when developing logistic regression models discussed in Section 5.3.

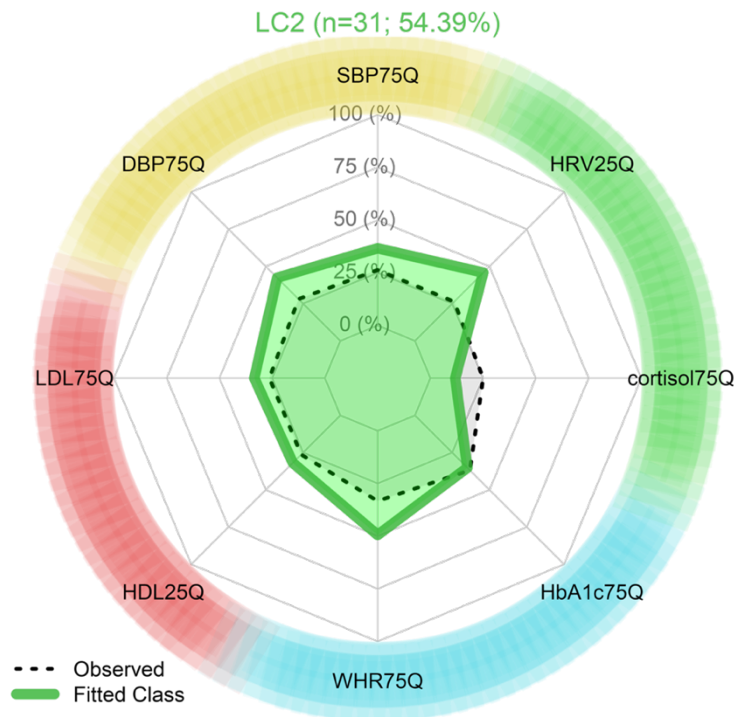


Figure 2: Visualizing results for LC2 from **Table 2:** LCA results for ALP using study-based thresholds when WHR is used as the obesity biomarker.

In contrast to LC1, the LC2 profile in **Figure 2** (relative to the observed profile) shows slightly elevated values in all biomarkers except cortisol and HbA1c. Therefore, we designated this class to be the elevated class. The number of subjects categorized into LC2 was 31 of the 57, which constitutes 54.39% of the firefighters from the study sample.

With our proposed method of developing allostatic load profiles, results from study-based thresholds with WHR as the obesity biomarker identified LC1 as the “less at-risk” profile, while LC2 was the “more at-risk” profile. The profiles of LC1 and LC2 highlight the advantage of using a profile in capturing the multivariate nature of allostatic load over the traditional allostatic load index. Next, we developed our allostatic load profiles using study-based thresholds and body mass index as the obesity biomarker.

Table 3: LCA results for ALP using study-based thresholds when BMI is used as the obesity biomarker.

Label	Class Size	Proportions by Biomarkers							
		SBPq75	DBPq75	BMIq75	HbA1cq75	LDLq75	cortisolq75	HRVq25	HDLq25
LC1	0.158	1.000	0.469	0.755	0.561	0.000	0.138	0.656	0.218
LC2	0.842	0.142	0.250	0.182	0.337	0.337	0.271	0.199	0.271
Prevalence	—	0.263	0.281	0.263	0.368	0.263	0.250	0.263	0.263

Note: “q75” reflects the corresponding variable’s 75th quartile, and similarly “q25” reflects the corresponding variable’s 25th quartile. This model had fit statistics as follows: BIC = 583.291 entropy= 4.564.

LC = Latent Class.

Based on results from **Table 3**, it is estimated that 15.8% of the study population belongs to class 1 while 84.2% of the study population belongs to class 2. **Table 3** also demonstrates that class 1 members are most likely to have elevated SBP, BMI and HRV compared to other biomarkers. Results in **Table 3** are better understood via **Figure 3** and **Figure 4**, which are shown next.

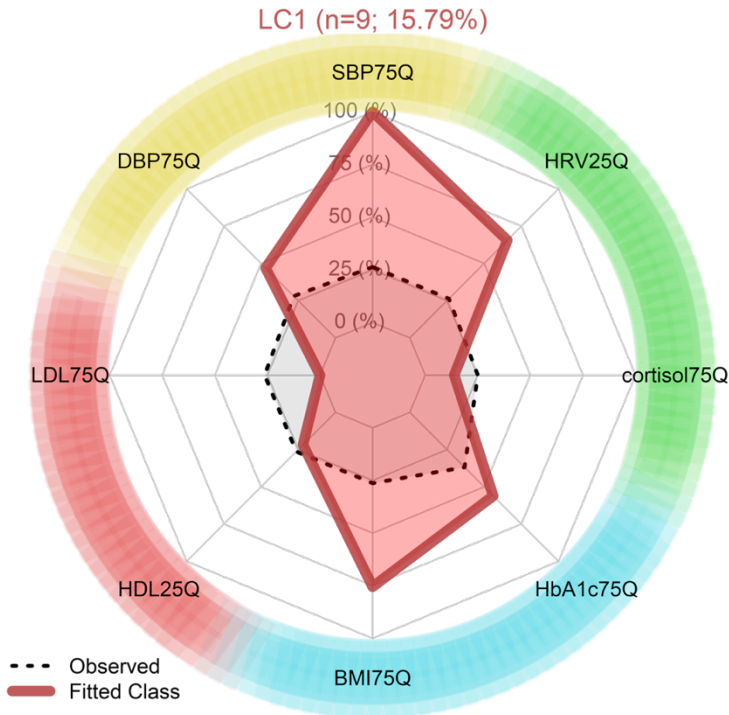


Figure 3: Visualizing results for LC1 from **Table 3**: LCA results for ALP using study-based thresholds when BMI is used as the obesity biomarker.

In **Figure 3**, the observed profile once again highlights the fitness of the sample of firefighters as the biomarker with the greatest amount of elevation was only around 30% and most were closer to 25%. In comparison to the observed profile, LC1 shows extremely elevated values in BMI and SBP, as well as elevated values in all other biomarkers except for LDL, HDL, and cortisol relative to the observed profile. Therefore, we designated LC1 as the elevated class. Only 9 of the 57, or 15.79% of the firefighters, were categorized into LC1.

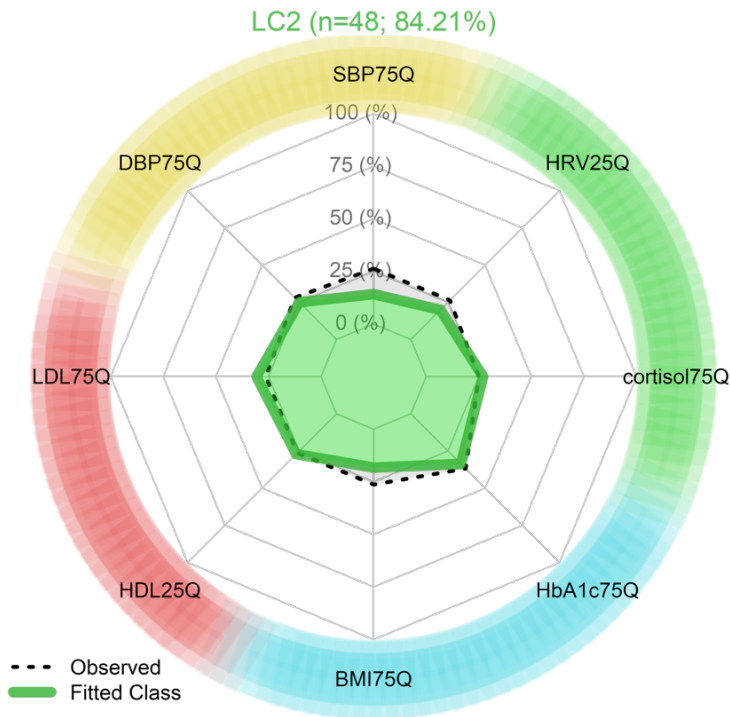


Figure 4: Visualizing results for LC2 from **Table 3**: LCA results for ALP using study-based thresholds when BMI is used as the obesity biomarker.

In contrast to LC1, **Figure 4** shows that most of the LC2 profile (outlined in green) falls within the observed profile, except for LDL, which very slightly exceeds the observed profile. Hence, this suggested LC2 be designated as the “normal” or non-elevated class. We can see that 48 of the 57, or 84.21% of the firefighters, were categorized into LC2. Once again, with our proposed method of using allostatic load profiles, results from study-based thresholds with BMI as the obesity biomarker identified LC2 as the “less at-risk” profile, while LC1 was the “more at-risk” profile.

5.2.2 ALP using a hybrid of Clinical- and Study-based Thresholds for Biomarkers: WHR versus BMI (Objective 1b)

Our initial goal was to create allostatic load profiles in two ways, first using study-based thresholds for biomarkers and second using clinical based thresholds. The clinical thresholds were established for the general population, and these thresholds were less likely to be representative of firefighters. When using clinical thresholds, we ran into issues with several biomarkers. Firstly, the clinical threshold for WHR is ≥ 1 for dichotomization (Bizik et al. 2013). In our sample of firefighters, the highest WHR was 0.63, still far below the clinical threshold. Similarly, the dichotomization for HbA1c, with a threshold of 6.2mmol/L (Bizik et al. 2013), resulted in only 2 of the 57 participants exceeding these thresholds. These small counts are likely due to the occupation of firefighting requiring a greater level of physical fitness in comparison to the general population (Song, Meng, István & Gu, 2020). Since none of the firefighters exceeded the clinical threshold for WHR, we decided to use study-based thresholds for WHR. Had we used the clinical cut-offs for WHR, this biomarker would not contribute to the model, and we would not be able to make any interpretations or recommendations for firefighters in regard to WHR. Furthermore, several biomarkers did not have firmly established clinical thresholds. Clinical thresholds from Bizik et al. (2013) did not include firmly established thresholds for LDL, HRV or cortisol. For these reasons, we decided to proceed with a hybrid model, in which clinical thresholds were used for SBP, DBP, BMI, HbA1c, and HDL, and study-based thresholds for WHR, LDL, HRV and cortisol. To begin, we developed allostatic load profiles using hybrid thresholds and WHR as the obesity biomarker.

Table 4: LCA results for ALP using hybrid thresholds when WHR is used as the obesity biomarker.

Label	Class Size	Proportions by Biomarkers							
		SBP-C	DBP-C	WHRq75	HbA1c-C	LDLq75	cortisolq75	HRVq25	HDL-C
LC1	0.508	0.589	0.308	0.561	0.000	0.204	0.206	0.373	0.168
LC2	0.492	0.000	0.000	0.000	0.073	0.319	0.321	0.098	0.000
Prevalence	–	0.333	0.175	0.317	0.032	0.254	0.259	0.254	0.095

Note: “q75” reflects the corresponding variable’s 75th quartile, and similarly “q25” reflects the corresponding variable’s 25th quartile. “-C” reflects that the variable was dichotomized using the clinically established threshold. This model had fit statistics as follows: BIC = 524.551 entropy = 3.648.
LC = Latent Class.

Based on the results from **Table 4**, it is estimated that 50.8% of the study population belonged to class 1 and 49.2% belonged to class 2. **Table 4** also shows that class 1 members are most likely to have elevated SBP, DBP and WHR compared to other biomarkers. These results when viewed in radar plots are discussed next in radar plots.

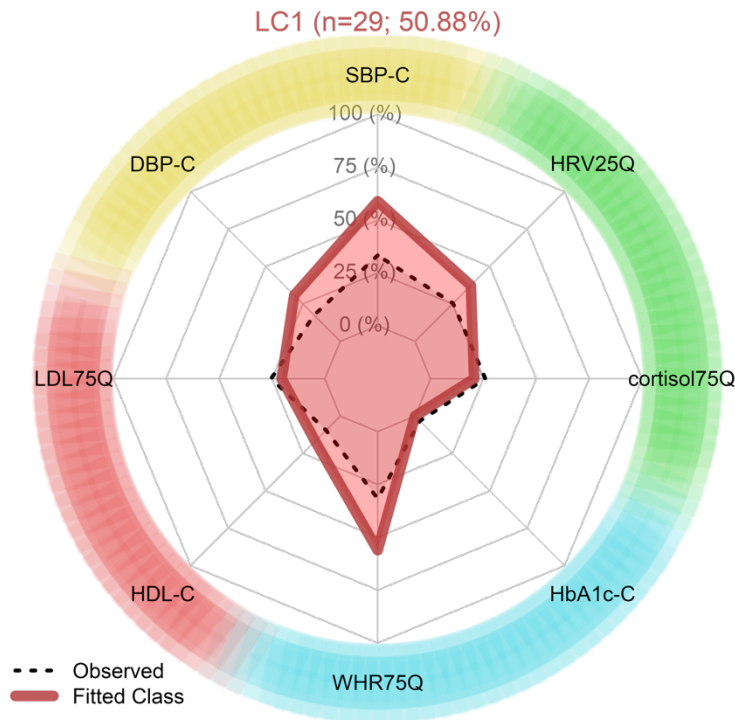


Figure 5: Visualizing results for LC1 from **Table 4**: LCA results for ALP using hybrid thresholds when WHR is used as the obesity biomarker.

In **Figure 5**, the observed profile highlights the differences between using clinical- and study-based thresholds for firefighters. As we can see, the LC1 profile shows elevations in SBP, WHR, DBP and HRV with respect to the observed profile. Hence, we designated LC1 to be the elevated class. Additionally, 29 of the 57, or 50.88% of the firefighters were categorized into LC1.

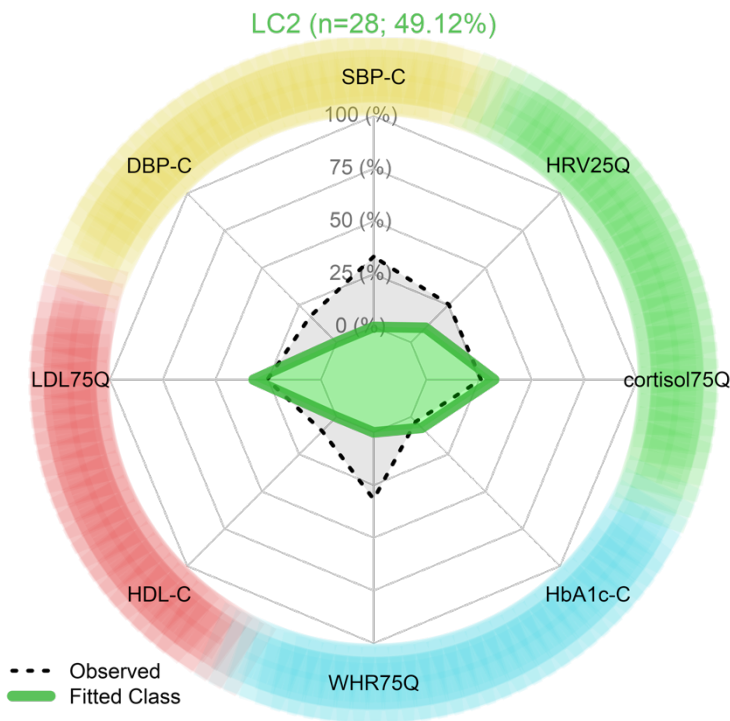


Figure 6: Visualizing results for LC2 from **Table 4**: LCA results for ALP using hybrid thresholds when WHR is used as the obesity biomarker.

In contrast to LC1, **Figure 6** shows LC2 (relative to observed profile) only shows a slight elevation in cortisol and LDL. Therefore, we designated LC2 to be the non-elevated class. Furthermore, we see that 28 of the 57, or 49.12% of the firefighters, were categorized into LC2. Next, we developed our allostatic load profiles using hybrid thresholds with BMI as the obesity biomarker.

Table 5: LCA results for ALP using hybrid thresholds when BMI is used as the obesity biomarker.

Label	Class Size	Proportions by Biomarkers							
		SBP-C	DBP-C	BMI-C	HbA1c-C	LDLq75	cortisolq75	HRVq25	HDL-C
LC1	0.365	0.824	0.397	0.906	0.000	0.109	0.261	0.336	0.163
LC2	0.635	0.000	0.023	0.744	0.053	0.352	0.257	0.198	0.049
Prevalence	–	0.333	0.175	0.810	0.032	0.254	0.259	0.254	0.095

Note: “q75” reflects the corresponding variable’s 75th quartile, and similarly “q25” reflects the corresponding variable’s 25th quartile. “-C” reflects that the variable was dichotomized using the clinically established threshold. This model had fit statistics as follows: BIC = 516.416 entropy= 3.577.

LC = Latent Class.

Based on the results from **Table 5**, we estimated that 36.5 % of the study population belonged to class 1, while 63.5% of the study population belonged to class 2. **Table 5** also demonstrates that class 1 members were most likely to have elevated SBP, DBP, and BMI compared to other biomarkers. Next, **Table 5** results are visualized in radar plots.

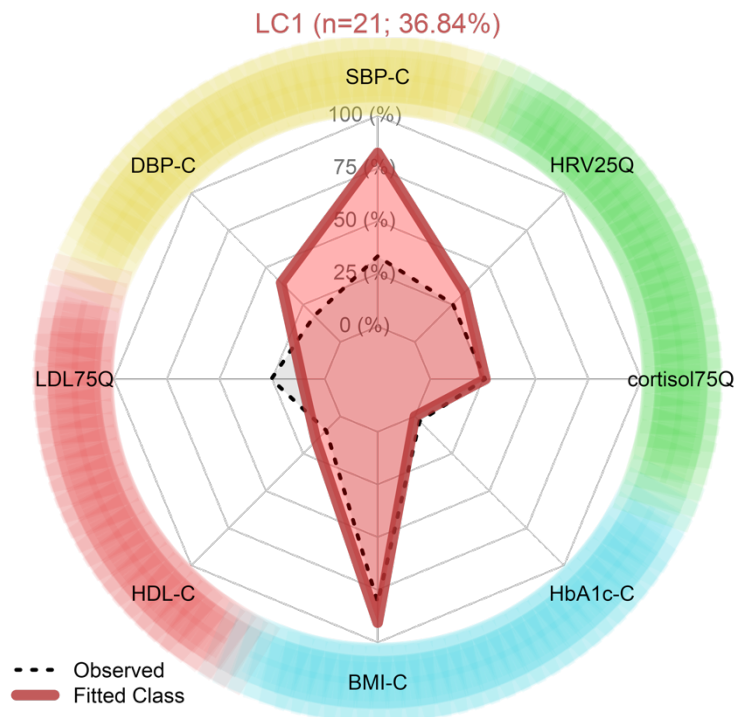


Figure 7: Visualizing results for LC1 from **Table 5**: LCA results for ALP using hybrid thresholds when BMI is used as the obesity biomarker.

In **Figure 7**, the observed profile highlights the differences between using clinical and study-based thresholds for firefighters, particularly for biomarkers such as BMI. As we can see, the observed profile shows that BMI elevation was over 75%. This is in stark contrast to the observed profile when using study-based thresholds as seen in **Figure 3** and **Figure 4**. Furthermore, when using BMI as opposed to WHR, we can see in the observed profile that close to 75% of the study sample had an elevated BMI. Given the major elevation in SBP, as well as elevations in DBP and HRV, we determined this to be the elevated class, with 21 of the 57, or 36.84%, of the firefighters designated to LC1.

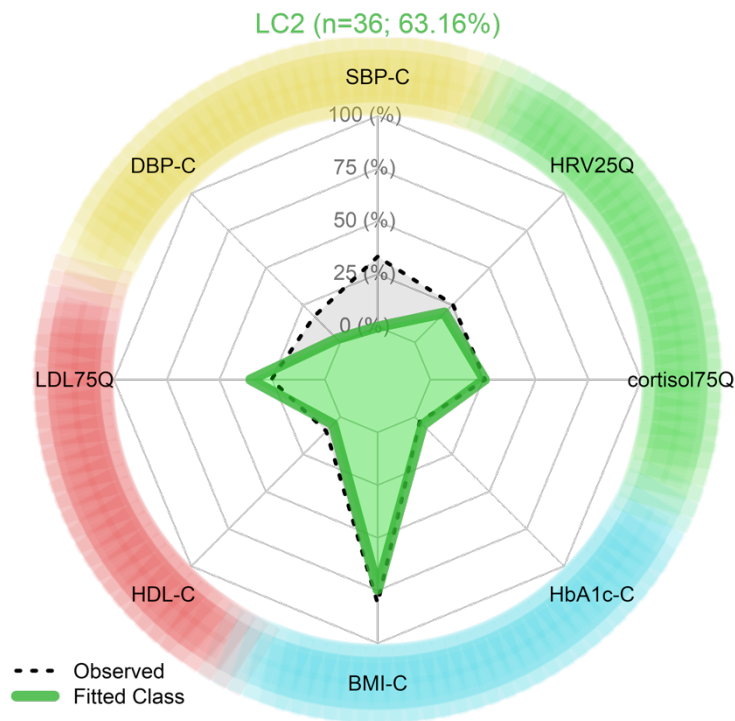


Figure 8: Visualizing results for LC2 from **Table 5**: LCA results for ALP using hybrid thresholds when BMI is used as the obesity biomarker.

In contrast to LC1, **Figure 8** shows LC2 (relative to the observed profile) had elevated values in LDL only, and this elevation is minor. Therefore, we designated LC2 to be the non-elevated or normal class and that 36 of the 57, or 63.16% of the firefighters, were categorized

into LC2. Lastly, with our proposed method of using allostatic load profiles, results from hybrid thresholds with BMI as the obesity biomarker identified LC2 as the “less at-risk” profile, while LC1 was the “more at-risk” profile for allostatic load. The profiles of LC1 and LC2 highlight the advantage of using a profile in capturing the multivariate nature of biomarkers over an allostatic load index.

Overall, when using clinical thresholds, we were able to capture two distinct profiles using BMI and WHR as separate obesity biomarkers in LCA models. However, the BMI versus WHR models showed profiles with high elevations, which was not expected in a sample of persons in good physical health.

5.2.3 Selecting an Optimal Model for ALP: BMI versus WHR and Study-based Thresholds versus Hybrid Thresholds (Objective 1c)

From Sections 5.2.1 and 5.2.2, we have 4 competing models for ALP because we have study-based thresholds versus hybrid thresholds for BMI versus WHR to measure obesity. To choose an optimal model, we used empirical metrics (BIC and entropy) and contextual metrics or impact (literature and interpretation of results). These metrics for the four models are summarized in **Table 6**.

Table 6: Summary of metrics used to assess the “best” model for ALP when considering study-based thresholds versus hybrid thresholds and within each, obesity measures BMI versus WHR.

LCA Model Type	BIC	Entropy	Contextual Pros and Cons
Study-based with BMI	583.291	4.564	Cons: BMI is not the best obesity biomarker for this sample and the BMI results do not make intuitive sense (see paragraph below).

Study-based with WHR	593.492	4.632	Pros: WHR will be better at reflecting obesity in this sample and the results make intuitive sense (see paragraph below).
Hybrid with BMI	516.416	3.577	Pros: The use of clinical thresholds highlights the differences between firefighters and the general population. Cons: Clinical thresholds are not applicable for a specialized group like firefighters. BMI is not the best obesity biomarker for this sample and the BMI results do not make intuitive sense (see paragraph below).
Hybrid with WHR	524.551	3.648	Pros: The use of clinical thresholds highlights the differences between firefighters and the general population. WHR will be better at reflecting obesity in this sample and the results make intuitive sense (see paragraph below). Cons: Clinical thresholds are not applicable for a specialized group like firefighters.

When determining whether to proceed with WHR or BMI as the obesity biomarker in the development of allostatic load profiles, several factors were considered. First, we looked at BIC and entropy. Based on results summarized in **Table 6**, BIC favours the model using hybrid thresholds with BMI as the obesity biomarker, while entropy favours the model using study-based thresholds with WHR as the obesity biomarker. Due to the disagreement between BIC and entropy for the optimal model for ALP, several additional factors were considered to help select the best model. It is important to acknowledge that the sample size of 57 participants was limited to a distinctness value of 0.63. Additionally, the existing literature explains that WHR is better at assessing fat distribution than BMI, which simply assesses overall mass. Consideration of mass is particularly important for firefighters because BMI cannot account for muscle mass and the literature suggests firefighters have higher muscle mass, especially in the lower body, than the general population (Song, Meng, István & Gu, 2020). Finally, the interpretation of fitted profiles from LCA also plays an important role when selecting the “best” model (Collins & Lanza, 2010,

p.148). The allostatic load profiles for study-based thresholds with WHR as the obesity biomarker, as shown in **Figure 1** and **Figure 2**, demonstrate that elevations are less drastic in all the biomarkers in the elevated profile. This observation was expected because the sample of firefighters should be in better health than the general population. Furthermore, when BMI was used as the obesity biomarker with the clinical cut-off values, we can see that most of the firefighters were incorrectly categorized as having “elevated” BMI values. Bearing all these considerations in mind, the LCA model using WHR as the obesity biomarker and the study-based thresholds was our optimal LCA model for allostatic load profiles for male firefighters from Waterloo Fire Rescue.

5.3 Investigating Associations between Allostatic Load Profiles (ALP) and Perceived Stress using Logistic Regression Models

5.3.1 Exploration of associations between perceived stress variables and ALP

In the initial study conducted by Igboanugo et al., (2022), the authors in their exploratory analysis considered 4 variables of perceived stress and social support: PSS-10, SOOS-14, MS-PSS, SSS-FF. Their investigation found none of these perceived stress variables to be associated with ALI. Our investigation with ALP, as summarized in **Table 7**, found only PSS-10 to be significantly associated with ALP, while SOOS-14, MS-PSS and SSS-FF did not show an association with ALP. Hence, as we proceeded with building regression models for ALP, PSS-10 was used as the measure of perceived stress.

Table 7: Summary results from 4 separate simple logistic regression models exploring association of each of the perceived stress variables with ALP.

Parameter	Estimate	Std. Error	OR	95% CI
PSS-10	0.128	0.062	1.136	(1.012, 1.299)
SOOS	0.044	0.064	1.045	(0.922, 1.192)
MS-PSS	-0.079	0.065	0.924	(0.809, 1.046)
SSS-FF	-0.106	0.066	0.899	(0.780, 1.013)

5.3.2 Investigating the Role of Confounding Variables in the Associations Between ALP and PSS

Since the literature does not provide a consensus on a defined set of confounders for the association between perceived and biological stress, we conducted exploratory data analysis for potential confounders as follows: age, length of service (LOS), smoking, sleep hours, exercise, and alcohol intake frequency. This list of variables is categorized into two domains such that age and LOS reflect aging and work experience, while the other variables represent the behavioural domain.

Table 8: ALP Regressed on LOS and Age in Separate Simple Logistic Regression Models.

Parameter	Estimate	Std. Error	OR	95% CI
LOS	0.024	0.033	1.024	(0.959, 1.097)
Age	0.047	0.035	1.048	(0.981, 1.126)

Neither LOS nor age is significantly associated with ALP (Table 8). Despite this lack of significance, literature suggest accounting for a variable from the aging and work experience domain as a control variable. We decided to proceed with LOS as a confounder over age for multiple reasons. First, in firefighting, one can reasonably assume the lowest possible age for a firefighter would be 25 years of age, given that in this sample, around 90% of the firefighters had a college degree or more education (Igboanugo et al., 2022). Knowing this information, LOS is

informative of both age and work experience, whereas with age one cannot ascertain work experience. Additionally, the literature suggests an association between length of service and severity of stress exposure over time (Makara-Studzińska, Wajda, Lizińczyk, 2020). For these reasons, it is important to consider LOS over age as a confounder when assessing the association between allostatic load and perceived stress.

Next, we investigated the role of behavioural confounders on ALP. Current literature suggests certain behaviours, especially smoking and alcohol consumption, play a role in both physiological and perceived stress (McEwen, 2000). While it is well established that smoking and frequent alcohol consumption impact biological markers such as blood pressure and obesity, they are also coping mechanisms that reflect individual stress perceptions and should therefore be addressed in analyses involving ALP.

Table 9: *ALP Regressed on Behavioural Confounders in Separate Simple Logistic Regression Models.*

Parameter	Estimate	Std. Error	OR	95% CI for OR
Smoking (Yes)	1.570	1.131	4.808	(0.709, 95.582)
Sleep Hours (<6)	-0.457	0.738	0.633	(0.140, 2.711)
Sleep Hours (>8)	0.172	0.963	1.188	(0.179, 9.712)
Exercise (No)	0.388	0.784	1.474	(0.325, 7.830)
Alcohol Intake (Any)	0.182	1.440	1.200	(0.046, 31.388)

None of the potential behavioural confounders were found to be statistically significantly associated with ALP. However, it is important to acknowledge the limited sample size of this study ($N = 57$) and that statistical significance (i.e., p-value) is a function of sample size. Hence, clinically significant associations may exist between some of these behavioural confounders and ALP, but such associations could not be detected in this study due to the small sample size. To

err on the side of caution, we will consider multiple variable logistic regression models where LOS will serve as a control variable for aging and work experience with and without behavioural confounders.

5.3.3 Selecting an Optimal Logistic Regression Model for the Association Between PSS-10 and ALP while Controlling for Confounders

To determine an optimal logistic regression model for assessing the association between PSS-10 and ALP, one avenue we pursued was to use statistical metric of the BIC, which is summarized below.

Table 10: Summary results from separate multiple variable logistic regression models exploring association of PSS with ALP while controlling for confounding variables

Model with predictors	BIC
PSS	81.915
PSS + LOS	85.677
PSS + Age	84.319
PSS + LOS + Smoking + Exercise	90.009
PSS + LOS + Smoking + Exercise + Sleep + Alcohol Intake Frequency	114.633

We found that three of these models: PSS, PSS + LOS, and PSS + LOS + all behavioural confounders are worthy of discussion. First, we determined that based on the literature, LOS would be the better aging and work experience confounder to proceed with as concluded in section 5.3.2. Furthermore, because there is no consensus in the literature on which confounders should be accounted for in the association between PSS-10 and allostatic load, it is important to account for all behavioural confounders as well. These three models are described and discussed next. The model equations corresponding to these three models are provided in Appendix B.

The parameter estimates from the model where binary ALP (from study based with WHR) was regressed on PSS-10 is shown in **Table 11**. This model suggests that the odds of being in LC2 (i.e., elevated profile) versus LC1 (normal or non-elevated profile) increase by 13.6% (OR 95% CI: (1.012, 1.299)) for each 1-unit increase in PSS-10 score. This finding is significant because the corresponding confidence interval is greater than 1.

Table 11: Logistic regression results from PSS regressed on ALPs created using study-based thresholds and WHR as the obesity biomarker

Parameter	Estimate	Std. Error	OR	95% CI for OR
(Intercept)	-1.809	1.003	0.164	(0.019, 1.062)
PSS	0.128	0.062	1.136	(1.012, 1.299)

The parameter estimates from the model where binary ALP (from study based with WHR) was regressed on PSS-10 while controlling for LOS are shown in **Table 12**. This model suggests that the odds of being in LC2 (i.e., elevated profile) versus LC1 (normal or non-elevated profile) increase by 13.4% for each 1-unit increase in PSS-10 score while controlling for LOS. This finding is significant because the corresponding confidence interval is greater than 1.

Table 12: Logistic regression results from PSS regressed on ALPs created using study-based thresholds and WHR as the obesity biomarker while controlling for LOS.

Parameter	Estimate	Std. Error	OR	95% CI for OR
(Intercept)	-2.024	1.093	0.132	(0.013, 1.006)
PSS	0.126	0.063	1.134	(1.009, 1.298)
LOS	0.018	0.035	1.018	(0.952, 1.094)

The parameter estimates from the model where binary ALP (from study based with WHR) was regressed on PSS-10 while controlling for LOS is shown in **Table 13**. This model suggests that the odds of being in LC2 (i.e., the elevated profile) versus LC1 (i.e., the normal or

non-elevated profile) increase by 19.2% for each 1-unit increase in PSS-10 score while controlling for LOS, Smoking, Sleep Hours, Exercise, and Alcohol Intake Frequency. This finding is significant because the corresponding confidence interval is greater than 1.

Table 13: Logistic regression results from PSS regressed on ALPs created using study-based thresholds and WHR as the obesity biomarker while controlling for LOS and behavioural variables.

Parameter	Estimate	Std. Error	OR	95% CI for OR
(Intercept)	-2.042	1.674	0.616	(0.003, 4.608)
PSS	0.176	0.075	1.192	(1.039, 1.400)
LOS	0.031	0.040	1.032	(0.956, 1.120)
Smoking Yes	2.307	1.220	0.100	(1.249, 223.981)
Sleep Hours (<6)	-0.750	0.867	0.473	(0.075, 2.498)
Sleep Hours (>8)	0.994	1.174	5.715	(0.297, 35.754)
Exercise No	-0.319	0.988	0.727	(0.096, 5.207)
Alcohol Intake (Any)	-1.107	1.546	0.331	(0.011, 9.913)

Note: for smoking the reference category was not smoking, for sleep the reference category was 6-8 hours per night, for exercise the reference category was >10 mins in the past 10 days, for alcohol intake frequency the reference category was never drinking.

The association between PSS-10 and ALP maintained statistical significance and directionality regardless of the mix of confounders (ageing, work experience or behavioural) included in the model. In all cases, a higher PSS-10 score was positively associated with the odds of being in the “elevated risk” ALP group. Across the three models, the odds ratios for the association between PSS-10 and ALP were approximately 1.13 and 1.20 (as seen in **Table 11**, **Table 12** and **Table 13**). Such results suggest the confounders were acting almost independently of PSS-10.

When selecting the “best” model between these logistic regression models, we had to consider how the sample size of our data could have affected our findings in terms of potential confounders in the association between PSS-10 and ALP. As stated in section 5.3.2, statistical

significance (p-value) is influenced by sample size. Furthermore, when examining our sample data, it was important to consider the low frequency of “risky” behaviours. Given that firefighters are required to maintain good physical health for their jobs, we found that few of our sample participants smoked, over-consumed alcohol, under-slept or eschewed physical activity, thus rendering the behavioural confounders secondary to the primary goal of the thesis, which was to employ the statistical technique of creating ALP and examine the association between PSS-10 and ALP. This thesis was not designed to establish a set of confounders in the association between PSS10 and ALP. Given our small sample size, the low frequency of “risky” behaviours in our sample, and our objectives, we determined that the “best” model was the model which regressed ALP on PSS-10 while controlling for LOS. In essence, our rationale for the inclusion of LOS in the model was the existing literature strongly supporting its impact on how firefighters perceive stress, as previously explained in Section 5.3.2.

Chapter 6: Discussion

6.1. Choosing an Obesity Biomarker

We investigated the influence of different obesity markers, WHR versus BMI, when developing allostatic load profiles. To determine what obesity biomarker is more appropriate for assessing allostatic load profiles in our sample of firefighters, the following factors were taken into consideration: suggestions from existing literature, characteristics of the study sample, model fit statistics, and interpretability of results. Existing literature highlights the differences in the body composition of firefighters and the general population. Because firefighters are required to pass physical fitness tests, they typically have a high muscle mass (Song, Meng, István & Gu, 2020). As previously stated, statistical tests pointed to waist-to-hip ratio as being a better measure of obesity. Furthermore, the allostatic load profiles obtained through WHR, as opposed to BMI, follow a healthy pattern that is expected in firefighters (Song, Meng, István & Gu, 2020). Since firefighters must maintain strict physical standards to meet the demands of their job, we would expect that even in the “elevated” allostatic load profile(s), the proportion of firefighters exceeding biomarker thresholds should not be exceedingly high. These findings highlighted the importance of not relying solely on model fit statistics, especially when they disagree in data with small sample size. Contextual interpretation of results, particularly the ease of interpretation and how well the results fit with current literature, is very important. The key finding of this investigation was that it is important to consider characteristics of the study population and sample data when making decisions around which physiological biomarkers to use in the computation of ALP. Our investigation found that the choice of an obesity biomarker can greatly influence the allostatic load profiles, especially in data with small sample sizes.

6.2. Challenges in Applying Clinical Thresholds and Overcoming Them

Our study investigated two dichotomization methods for biomarkers – study-based and clinical thresholds. Challenges arose when applying clinical thresholds because these thresholds are established using the general population, which is likely to have physiological biometrics different from firefighters due to the nature of the job of firefighting. For example, when using clinical thresholds, $WHR \geq 1$ was an indicator for “high risk” and none of the subjects in our study fell into this category, which is not surprising because firefighters are generally in better physical health compared to the general population. Therefore, one cannot build a latent class model for firefighters with the clinical threshold for WHR. Our challenges in applying clinical thresholds to firefighters should encourage future researchers to examine the applicability of the thresholds they use when researching specialized populations.

6.3. Perceived Stress as a Predictor of Physiological Stress

In our study, we set out to determine whether perceived (subjective) stress would be associated with physiological (objective) stress measured using ALP. While one may reasonably expect that subjective stress would be associated with physiological stress, the current literature on the topic is limited and inconclusive. For example, Dorsey et. al. (2022) reported that some studies found weak associations, where 16% of the variance in HCC levels were explained by the perceived stress scale, while other studies found that PSS was not significantly correlated with hair cortisol levels. These discrepant results arise because many factors play a role in individual perceptions of stress. For instance, resilience describes an individual’s ability to cope with negative events and emotions such as stress. Factors such as resilience can alter an individual’s subjective impression of the stress they face, thereby changing the expected positive association between subjective and objective stress. The phenomenon that explains inconsistencies between

subjective and objective stress is called ‘psychoendocrine variance’. Psychoendocrine variance suggests that while subjective experiences of stress such as nervousness should be coupled with an increased physiological stress response such as increased cortisol levels, this relationship is not always as clear as would be expected (Scholtz et. al, 2008). This inconsistency could be due to a lag of endocrine responses behind psychological responses.

Other reasons may also explain the divergence between subjective and objective stress. For example, the initial study by Igboanugo et al. (2022) did not find a significant association between any of the perceived stress scales and allostatic load when it was summarized via ALI. This could be explained by self-reporting bias in perceived stress measures (such as PSS-10) and the use of the ALI. As stated earlier, ALI, as a single score, is incapable of capturing the multivariate nature of allostatic load. In our study, we found a significant association between perceived stress (measured using PSS-10) and allostatic load when assessed via ALP. Furthermore, we demonstrated the association between PSS-10 and ALP maintained both significance and directionality after the inclusion of potential confounders (Section 5.3.2). As a result, our findings suggest that another possible source of the discrepancies in the literature regarding the association between subjective and objective stress may be due to how stress and/or its effects are evaluated.

6.4. Strengths, Limitations and Recommendations

This thesis makes several important contributions related to measuring allostatic load and its predictors, particularly in specialized groups. Firstly, the key finding of this thesis was the demonstration of an association between perceived stress and the cumulative biological effects of this stress, which was unaffected by the inclusion of behavioural, biological, and other potential confounders. Previous studies have failed to consistently demonstrate a strong association

between subjective and objective stress (Igboanugo et al., 2022; Dorsey et. al., 2022). This inability may be due to the design of measures of objective stress as opposed to issues with self-reporting subjective stress. We recommend that future researchers consider using ALPs, rather than the ALI, to more accurately capture the multivariate nature of allostatic load.

Additionally, because the use of ALP allows us to examine the true multivariate nature of allostatic load by showing how a combination of biomarkers is associated with perceived stress, the methodology of creating ALP – as described in this thesis – could have downstream implications for informing and creating better targeted interventions. For example, as seen in one of our findings, the ALP with elevated cortisol only was associated with perceived stress, so interventions such as stress reduction techniques can be provided to firefighters to reduce cortisol levels.

Furthermore, dichotomizing biomarkers through study-based thresholds and clinical thresholds highlighted the need to employ study-based thresholds for specialized populations, which may differ from the general population. Not only did we find that clinical-based thresholds were largely unable to capture the health biometrics of the specialized population, but we also demonstrated that empirical tests favoured the use of study-based thresholds for our study sample.

Notwithstanding the aforementioned contributions, this thesis is not without limitations. The first limitation we faced came from the use of a pre-existing data set. The sample size for this research was fixed at 57 after employing the exclusion criteria and our latent class models/inferences would therefore be subject to a power of at least 70% for a two-class LCA model and distinctness of 0.63. As previously noted, this sample size limitation led to

disagreement in model fit indices, which required us to rely more heavily on existing literature and contextual interpretation when making model selections.

Another limitation was the exclusion of female subjects from analyses. Due to biological, psychological, and sociological differences between males and females, our findings may be less applicable to female firefighters. A recent study by Longpre-Poirier et al. (2022) found that elevated allostatic load index in females was more strongly associated with immune system dysfunction, whereas in males it was associated more with cardiovascular issues. This suggests that females could have different elevations in key biomarkers for measuring allostatic load. As such, the allostatic load profiles observed in male firefighters may not be applicable or useful when examining female firefighters.

Finally, the population measured in the original study by Igboanugo et al. (2022) is a very specific population that encompasses firefighters in Waterloo Fire Rescue only. The City of Waterloo is largely suburban with some more densely populated urban areas and many rural areas. The occupational hazards and stressors faced by rural firefighters could be different from those faced by firefighters working in larger urban settings. As such, the findings for firefighters in Waterloo Fire Rescue may only apply to firefighters in similar geographical working environments.

Even though this study has limitations, they are not due to our methodological approach of developing ALP as a multivariate measure for allostatic load and its associated factors. Overall, we recommend further research to build upon our findings regarding the use of ALP and study-based thresholds to calculate allostatic load in specialized groups such as those where understanding and managing stress are paramount to safety and efficacy (e.g., first responders,

such as law enforcement officers and emergency medical service providers, military personnel, etc.).

Chapter 7: Conclusions

This thesis established a relationship between allostatic load profiles and perceived stress in firefighters. The perceived stress scale was associated positively with being in the “elevated” group for allostatic load, maintaining directionality and significance with or without controlling for confounding variables. Length of service was the most relevant control variable to consider for our study sample when looking at previous studies (Igboanugo et al., 2022), as well as literature supporting the importance of length of service on firefighter stress (Makara-Studzińska, Wajda, Lizińczyk, 2020). The findings from multiple regression models in the thesis repeatedly showed that firefighters who perceive their stress higher are more likely to be in a higher state of elevated allostatic load.

This thesis demonstrates that our finding of a significant association between PSS-10 and allostatic load, evaluated through latent class profiles, may make ALP worthy of investigation in future studies. Given our promising results from use of ALP, it may also be worthwhile to revisit previous studies where ALP is used a multivariate measure to summarize allostatic instead of the traditional (univariate) measures like ALI or singular biomarkers.

Our investigation with applying clinical threshold for our objectives presented us with more problems than solutions. Additionally, the use clinically established thresholds in our objectives yielded results that were not reflective of the study population. This demonstrates the need to use study-based thresholds in unique populations such as firefighters, who may have drastically different health metrics compared to the general population from which clinical thresholds are created. Furthermore, it is important to consider that clinical thresholds are often used as diagnostic tools by physicians to assess pathology in patients. Allostatic load on the other hand, is not measured for diagnostic purposes, but rather to better understand how chronic stress

exposure impacts individuals or groups of people. For this reason, it may not be sensible to apply diagnostic thresholds when measuring allostatic load.

In summary, it is important for further research to apply both allostatic load profiles and study-based thresholds when examining stress in unique populations. This study highlighted not only the relationship between perceived stress and physiological stress in firefighters, but also the need to use specialized thresholds to assess their physiological stress. There are many other populations and contexts where our methodology could be applied to better assess their stress and lessen the dangers they face in their occupations. For example, law enforcement officers could also benefit from a multivariate tailored analysis of their allostatic load and its association with perceived and occupational stress. Analyses similar to the ones conducted in this study could also be applied to first responders, military personnel, and to persons in other occupations such as pilots, loggers, and underwater welders, among others, where stress is high and safety is paramount.

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Appendix A: The Perceived Stress Scale (PSS)

Perceived Stress Scale

A more precise measure of personal stress can be determined by using a variety of instruments that have been designed to help measure individual stress levels. The first of these is called the **Perceived Stress Scale**.

The Perceived Stress Scale (PSS) is a classic stress assessment instrument. The tool, while originally developed in 1983, remains a popular choice for helping us understand how different situations affect our feelings and our perceived stress. The questions in this scale ask about your feelings and thoughts during the last month. In each case, you will be asked to indicate how often you felt or thought a certain way. Although some of the questions are similar, there are differences between them and you should treat each one as a separate question. The best approach is to answer fairly quickly. That is, don't try to count up the number of times you felt a particular way; rather indicate the alternative that seems like a reasonable estimate.

For each question choose from the following alternatives:

0 - never 1 - almost never 2 - sometimes 3 - fairly often 4 - very often

- _____ 1. In the last month, how often have you been upset because of something that happened unexpectedly?
- _____ 2. In the last month, how often have you felt that you were unable to control the important things in your life?
- _____ 3. In the last month, how often have you felt nervous and stressed?
- _____ 4. In the last month, how often have you felt confident about your ability to handle your personal problems?
- _____ 5. In the last month, how often have you felt that things were going your way?
- _____ 6. In the last month, how often have you found that you could not cope with all the things that you had to do?
- _____ 7. In the last month, how often have you been able to control irritations in your life?
- _____ 8. In the last month, how often have you felt that you were on top of things?
- _____ 9. In the last month, how often have you been angered because of things that happened that were outside of your control?
- _____ 10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?