



## Review article

## Towards a biochemical approach to occupational stress management

Gareth L. Shackleton\*

BioTune Ltd, UK



## ARTICLE INFO

## Keywords:

Metabolomics  
Occupational stress  
Systems biology

## ABSTRACT

Given the immense and growing cost of occupational stress to society through lost productivity and the burden to healthcare systems, current best practices for detecting, managing and reducing stress in the workplace are clearly sub-optimal and substantially better methods are required. Subjective, self-reported psychology and psychiatry-based instruments are prone to biases whereas current objective, biology-based measures produce conflicting results and are far from reliable. A multivariate approach to occupational stress research is required that reflects the broad, coordinated, physiological response to demands placed on the body by exposure to diverse occupational stressors. A literature review was conducted to determine the extent of application of the emerging multivariate technology of metabolomics to occupational stress research. Of 170 articles meeting the search criteria, three were identified that specifically studied occupational stressors using metabolomics. A further ten studies were not specifically occupational or were of indirect or peripheral relevance. The occupational studies, although limited in number highlight the technological challenges associated with the application of metabolomics to investigate occupational stress. They also demonstrate the utility to evaluate stress more comprehensively than univariate biomarker studies. The potential of this multivariate approach to enhance our understanding of occupational stress has yet to be established. This will require more studies with broader analytical coverage of the metabolome, longitudinal sampling, combination with experience sampling methods and comparison with psychometric models of occupational stress. Progress will likely involve combining multi-omic data into a holistic, systems biology approach to detecting, managing and reducing occupational stress and optimizing workplace performance.

## 1. Introduction

Stress, and stress-related disease, is costing western economies trillions with hospitalization and lost productivity rising every year [1, 2]. Workplace stress costs companies billions in lost workdays, disengagement and declining productivity [3, 4]. There are four likely reasons that organizations have been unable to tackle this problem. Firstly, methods for detecting stress sufficiently early, may be sub-optimal [5]. Secondly, methods for managing stress, or the application of them, may be inadequate [6]. Thirdly, the issue may be under-appreciated and thus given low priority by organizational managers [7, 8]. Finally, inter-individual differences in biological stress responses mean we must titrate stressors and stress management according to individual needs and this is challenging using current tools [9, 10, 11].

In the occupational setting, current best practice for detecting work-related stress involves psychiatric, psychological or sociological outcome measures that are typically self-reported, subjective questionnaires. A systematic literature review of interventions to reduce sickness

absence [12], demonstrated that psychological distress, anxiety, depression and emotional exhaustion were frequently assessed using instruments such as the SCL-90 (an assessment of ninety symptoms associated with the most common psychiatric disorders), various versions of the General Health Questionnaire (GHQ), the Maslach Burnout Inventory (MBI) and a number of other clinical psychiatric instruments. Given the taboo of mental illness, and biases introduced by a respondent's own agenda, psychiatry-based self-reported tools may produce confounding results. Specifically, the SCL-90R instrument has been shown to be more valid in a patient-therapeutic setting than as a descriptive tool in the general population [13].

There are three main psychological models of occupational stress the Job-Demand-Control and Support model (JDCS), the Effort Reward Imbalance Model (ERI) and the Organisational (In)justice model (OI). The JDCS model [14, 15] measures three dimensions: psychological job demands, decision latitude (job control) and social support at work. Jobs that are characterized as high demand, low control and have low social or supervisory support are hypothesized to put employees at risk of

\* Corresponding author.

E-mail address: [garethshackleton@biotune.co](mailto:garethshackleton@biotune.co).

stress-related ill health and disease and associations have been made with biomarkers of cardiovascular disease [16].

The theoretical approach of the ERI model is the concept that stress occurs when efforts are out of proportion with perceived rewards [17]. This model has been useful in identifying overcommitment in highly engaged employees, which may lead to conditions of high effort/low reward and eventually to burnout and disengagement. A number of studies have found associations between ERI and biological markers of stress such as cortisol [18] and heart rate variability [19] Increased risk of developing metabolic syndrome and cardiovascular disease has also been associated with ERI [20].

The OI model measures the extent to which employees perceive workplace procedures, interactions and outcomes to be fair [21]. In this model there are two main dimensions: relational, or inter-personal, and procedural justice. Relational justice refers to the extent supervisors consider their employees' viewpoint and take steps to deal with subordinates in a fair and truthful manner. Procedural justice involves the fairness of formal decision-making procedures. At least one study has demonstrated a decrease in heart rate variability as a consequence of altered autonomic control related with organizational injustice although the result was not statistically significant [22].

Overall, a systematic review of the literature available at the time demonstrated that there was a general association between these psychological models of stress and biomarkers of stress (cortisol, catecholamines, HRV, prolactin, testosterone) but that there were inconsistencies in the results and the effects were not clear cut [23].

Studies that have attempted to identify objective, biological measures of occupational stress tend to look at correlations with the subjective instruments reviewed above, contributing to difficulties in interpreting biological information and potentially inhibiting the adoption of stress biomarkers in the workplace [23]. Thus, cortisol levels, one of the most commonly measured biological markers of stress, were not found to be associated with perceived stress state [24] or task unpleasantness [25, 26], or were inversely correlated with perceived stress in males and not correlated in females [27]. In a meta-analysis of 208 studies published in 2002, Dickerson and Kemeny found elevated cortisol associated with various types of acute stress, although the effect size varied considerably depending on the nature of the stressor [28]. In fact, some psychological stressors did not appear to elicit a cortisol response at all, consistent with the findings of others [29]. Interventions to reduce ERI were not associated with decreased levels of cortisol, although salivary amylase (a measure of the sympathetic nervous system) was decreased by the intervention [30]. On the other hand, increases in salivary cortisol in response to an experimentally induced acute stressor was associated with a background of high role uncertainty [31]. The kinetics of cortisol release, circulation and elimination are complex [32, 33, 34], as are those of other putative biomarkers [35], which may add to the difficulties of correlating individual biological measures with psychological instruments of stress.

Burnout is broadly considered to be one of the proximate diseases of long-term stress and is defined as "a syndrome conceptualized as resulting from chronic workplace stress" in the WHO's International Classification of Disease [36]. In a systematic review of biomarkers in occupational burnout, up to and including 2008, 31 articles involving 38 biomarkers met the inclusion criteria [37]. These biomarkers represented the hypothalamus-pituitary-adrenal axis (HPAA), the Autonomic Nervous System (ANS), the immune system, antioxidant defenses, sleep, metabolism and hormones other than stress hormones. None showed promise as potential biomarkers of burnout. A more recent narrative review of biomarkers in burnout, including studies reported up to 2018, came to a similar conclusion: "existing research cannot confirm reliable endocrinological and immunological markers of burnout" [38]. Subsequently, Traunmuller et al., have found that burnout was associated with physiological changes such as decreased heart rate variability, increased cortisol and increased blood pressure [39], but that there were two populations of burnout subjects: one with physiological changes and one

without. Psychological classifications such as burnout, much like the current classification of psychiatric diseases, tend to represent catch-all categories. This study illustrates that such classifications may need further segmentation and development of new biomarkers of occupational stress may facilitate such an objective.

The nature of the stress response is the activation or suppression of almost every tissue in the body in a tightly orchestrated answer to the demands, or perceived demands, placed on the organism by the stressor. As the demands are likely to be stressor dependent, it should not be expected that single components of the stress phenotype, such as cortisol or adrenaline, will be predictive, particularly if time courses are not covered carefully following exposure to acute, intermittent or chronic stressors. The coordinated responses of multiple biometrics over time are hypothesized to be more predictive. Therefore, methods capable of measuring multiple diverse biological endpoints are required.

To date, multi-analyte approaches have tended to concentrate on stress as a precursor of disease. One series of studies designed to operationalize allostatic load, emphasized metabolic syndrome markers such as BMI, waist-hip ratio or glycated hemoglobin [40, 41, 42, 43]. This emphasis on disease etiology was reflected in the approach taken to data analysis. Ten markers were measured in total and allostatic load was calculated by summing the number of parameters for which the subject fell into the highest-risk quartile of the study cohort - that is highest risk of developing cardiovascular disease e.g. elevated cortisol, high total cholesterol or high waist-to-hip ratio. The allostatic load score for any individual, or group of individuals, was therefore a relative risk factor for that particular study cohort and not a globally applicable allostatic load score. While this body of work leads the way for multi-marker investigations, objective (biological) measures of stress, useful for the prospective and proactive management of stress, are more likely to be associated with the proximate causes and responses to stress, rather than association with disease.

The development of genomic, transcriptomic, proteomic and metabolomic technologies coinciding with a massive increase in computing capability to facilitate data analysis, has brought with it the opportunity to move away from a reductionist, univariate approach towards a multivariate view of biology [44] and with it the opportunity to develop more objective, biological methods to detect and diagnose broad physiological phenomena such as stress responses. Omics technologies promise breakthroughs in biomarker discovery [45, 46, 47] and the identification of a small number of analytes suitable for biomarker assay validation is the aim of the majority of studies. Developing reproducible models based on dynamic multivariate patterns rather than a small number of analytes, is less researched, but is arguably more likely to be successful [44] in the stress research field because of the pleiotropic nature of the stress response.

A full review of genomic, transcriptomic, proteomic and metabolomic analyses in occupational stress is too broad to be covered in one paper. Ironically then, this review will focus solely on the use of metabolomics in occupational stress research. The ultimate goal of developing metabolomics methodologies in this field will be to guide employers towards individualized work patterns for employees to optimize their performance while minimizing the long-term deleterious effects of chronic stress.

## 2. What is metabolomics?

Just as genomics is the identification of the total complement of genes of a population and proteomics the identification of the entire complement of proteins, metabolomics is the identification of the entire metabolic composition of a cell, tissue or organism at any given moment in time. The basis for metabolomics is the concept that the concentration of metabolites in the body is representative of the overall physiological status of the organism [48]. It therefore has the potential to revolutionize the study of human health and conditions that perturb human health, such as occupational stress. In the last twenty years, metabolomic studies have created

new understanding of human health and aided in the identification of previously unpredicted biomarkers for human disease and therapies [49].

Metabolomic studies assay thousands of small molecules in cells, tissues, organs, or biological fluids and create metabolic “fingerprints” using multivariate statistical methodology. However, while it is possible to determine the whole genome, it is not yet possible to measure the whole metabolome. Importantly, while we know how many human genes there are, we don't yet know how many human metabolites there are. In fact, human metabolites are not just of human genetic origin, but also from the microbiome and from the diet and environment in general. This is both a drawback of metabolomics, but also a strength, as it reflects the complete biological state of an individual and the interaction between that individual, its genome and the environment [50].

One of the challenges of metabolomics is how to detect and quantify so many molecules with such different physicochemical properties. Aliphatic compounds behave differently to aromatics, amines to carboxylic acids. Phosphates, sulphates, sugar conjugates and all manner of ionizable and un-ionizable functional groups have to be accommodated for the detection, identification and quantification of metabolites. While a gene or gene transcript is made up of four similar components and a protein is made up of twenty, connected in a very predictable and linear fashion, small molecule metabolites can contain fifty or more functional groups connected in numerous ways. Two methodologies that can deal with some of this chemical diversity, nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS), are primarily used in untargeted metabolomic studies, while electrochemical detection (ECD) has proven useful for targeted neurotransmitter analysis [51]. Data from multiple platforms can be combined to give a broader coverage of the metabolome, although this complicates the data analysis and quality control.

The use of metabolomics in the occupational setting has precedent. The technique has been used extensively in toxicological studies [52, 53, 54], leading to the use of metabolomics in occupational exposure assessment [55, 56].

To summarize, stress is a complex state with multiple triggers and affecting multiple systems in the body. To manage occupational stress more effectively it is necessary to take a multivariate approach that objectively measures the plethora of physiological and biochemical changes that occur in response to acute, intermittent and chronic stressors. Metabolomics takes a broad, multivariate snapshot of the total biochemical composition of the body at any moment in time and has been used extensively to probe the effects of stressors such as environmental toxins or pharmaceutical overdose. It therefore has promise as a post-genomic methodology to aid in the understanding and management of occupational stress. The aim of the current review is to evaluate the current application of metabolomics in the study of occupational stress. This review focuses solely on human studies, although a number of metabolomic studies in animal models of stress have been reported [57, 58, 59, 60].

### 3. Materials and methods

A review of the literature was conducted on the application of metabolomics in occupational stress research. An initial PubMed search using the search term: (metabolomics OR metabonomics OR “metabolic profiling”) AND (“workplace stress” OR “occupational stress”) for all fields, returned sixty references between 2003 and 2020 out of 28,527 for the (“workplace stress” OR “occupational stress”) search term. The date range was chosen as there were no references matching the combined search term before 2003. A search of the BASE database ([base-search.net](http://base-search.net)) using the search string “occupational stress” OR “workplace stress” returned 11,292 records between 2003 and 2020, one of which was retained when the search string: (metabolomics OR metabonomics OR “metabolic profiling”), was added (using AND logic to append to the original search). Finally, a search of Google Scholar using the same search string and period returned 88 records. Beyond this systematic search, citations from the metabolomics literature revealed 21 additional

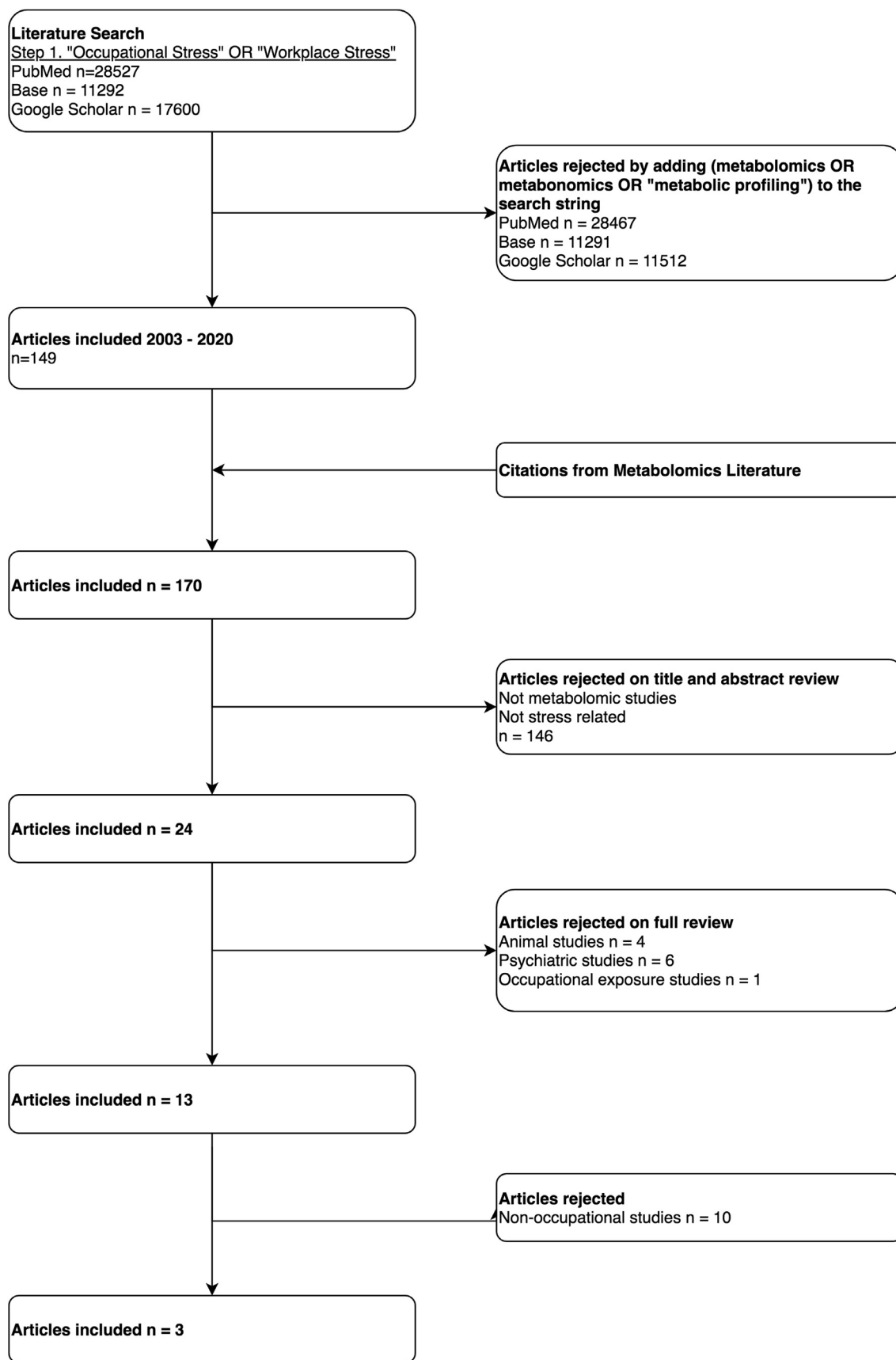
studies. The titles and abstracts of these 170 articles were reviewed manually to create a final body of research of direct relevance to the application of metabolomics to occupational stress.

## 4. Results

### 4.1. Literature search

The search strategy and the stepwise outcomes are outlined in [Figure 1](#). Manual review of the titles and abstracts of the 170 articles identified by the literature search revealed three studies of direct relevance: one study that examined the effect of shift work on the metabolome [61], one that examined the effects of mental fatigue (caused by a stressful occupation - air-traffic control) [62] and a third that investigated lifestyle stressors, including those related to work [63].

A number of other studies were identified that were of peripheral or indirect relevance. There were two studies that examined the clinical conditions of exhaustion disorder [64] and chronic fatigue syndrome [65], respectively. These were considered of peripheral significance in relation to mental fatigue and burnout at work, but as they were based on clinical diagnoses of unspecified origin, they were excluded from the review. A third study investigated the effect of deployment on the metabolome of service personnel [66]. While this may represent a specific case of occupational stress, the focus of the paper was on metabolomic changes caused by occupational exposure to environmental pollutants. Most of the changes observed were ascribed primarily to exposures to insecticides, herbicides and pollutants, such as oxidative stress and mitochondrial disruption, or to changes in physical conditioning, such as keratan sulphate. An additional confounding factor was the unknown gender mix in the case and control groups of the study, which was reflected in differences in sex hormones between groups and may have resulted in other metabolomic differences between groups. The results of this study were not therefore included in this review. A study into Post-Traumatic Stress Disorder (PTSD) was identified [67] and considered because of its relevance to certain occupations such as the armed forces, police, hospital workers, prison officers, etc. However, it was eventually excluded from the review because it involved clinical diagnoses of unspecified etiology. Two studies were identified that examined the effect of stress management interventions on the metabolome. One investigated the effect of Cognitive Behavioral Therapy (CBT) on psychological wellbeing of obese subjects with self-reported stress symptoms [68]. The reduction in perceived stress was not statistically significant, although there was an improvement in psychological flexibility as it pertains to weight-related difficulties (measured using the AAQW psychometric instrument) and heart rate variability, a measure of autonomic nervous system tone that has been used as a marker of stress. The AAQW score was associated with changes in the plasma metabolome, primarily in the phosphatidylcholine profile and generally weak associations were found between stress and lipid components of the plasma metabolome, although the causal relationship between psychological flexibility, stress and metabolite changes was not determined. In another study, the plasma metabolome was measured to determine the effects of a six-day Ayurvedic (“Perfect Health”) intervention [69]. Significant reductions were detected in the circulating levels of twelve phosphatidylcholines following the intervention, which may have been due to the use of hypolipidemic herbal medicines that the subjects took during the procedure but is more likely due to the dietary restriction of dairy, meat and eggs. Although these studies were not included in the review because they did not study occupational stress per se, they demonstrated the potential for stress management interventions to be detected in the metabolome. They also demonstrate the potential for confounding factors to interfere with metabolomic studies of occupational stress. Other potential confounding factors studied using metabolomics have been ageing [70], health status [71, 72], sleep deprivation [73, 74] and psychiatric conditions [75, 76, 77, 78, 79, 80] such as schizophrenia and depression.



**Figure 1.** Literature Search Schematic. The initial search string in PubMed in its entirety was: ("occupational stress") OR ("workplace stress") AND ((metabolomics) OR (metabonomics) OR ("metabolic profiling")). After the initial search the search period was restricted to 2003 to 2020 as there were no relevant articles before 2003.

#### 4.2. Methodological comparison of metabolomic studies in occupational stress

Three studies were found that used metabolomics to investigate occupational stressors. The study of Sood et al. [63], investigating psychological stress caused by various lifestyle and occupational chore-based stressors, differed from the other two in that it measured the serum metabolome and utilized  $^1\text{H-NMR}$ .  $^1\text{H-NMR}$  is particularly well suited to metabolomics because it can be used on crude samples, such as diluted urine, without extraction steps that can change the relative concentrations of metabolites in the sample [52, 53]. A particular benefit of NMR is that the complete molecular structure of a metabolite can be elucidated, although formal identification would generally be confirmed with a reference standard. Quantitation is also relatively straight forward as the signal response correlates to the number of protons in the molecule. However, NMR is relatively insensitive compared to mass spectrometry or electrochemical detection. One of the challenges facing all analytical methods used for metabolomics is that they may be biased towards specific chemical motifs, which can emphasize certain pathways over others, e.g. energy or amino acid metabolism by NMR or lipid metabolism by GC-MS [54]. However, in stress-research, particularly in the study of the regulation of energy utilization by the HPA axis and glucocorticoids, this may be an advantage allowing broad targeting of carbohydrate and lipid metabolism.

Serum samples offer a snapshot of the metabolome at any given moment in time and collection can be standardized. However, Sood et al. did not specify or appear to standardize time of day for sample collection. NMR offers rapid analysis and relatively robust metabolite quantification. Sood et al. utilized a “constant peak at around 5.32ppm which was set to zero integral” to standardize signal intensity for metabolite quantification by peak area integration. Explicit identification of this peak would improve comparison with other studies.

In contrast, the study of Rotter et al. [61] into the effect of night-shiftwork and that of Chen et al. [62] into the effect of mental fatigue caused by a day of intense, cognitively challenging work (air-traffic control) both measured the urine metabolome using LC-MS. MS methods offer much greater sensitivity than NMR (several orders of magnitude) and a wide dynamic range for metabolite detection, identification and quantitation. Metabolite identification by MS is usually only partial in the absence of a synthetic standard or some prior knowledge of structure. Quantifying metabolite concentrations by MS is problematic due to matrix effects on the signal. The gold standard for MS quantification is to use isotopically labelled standards, which is only possible for known metabolites and limits the number of metabolites that can be fully quantified in a sample or analytical run. Although direct MS analysis of biological samples has shown some promise in metabolomic studies, using plasma ionization techniques to introduce the sample into the spectrometer [81], some separation of metabolites from the matrix and from other metabolites is usually preferred to facilitate identification and quantitation of a larger number of components. Liquid chromatography (LC), gas chromatography (GC) and capillary electrophoresis (CE) are the most common modes of analyte separation. GC-MS has been widely used in lipidomic studies [82] but cannot easily be used to measure non-volatile compounds without derivatization, which introduces another variable into the analysis that must be controlled. LC-MS is arguably the most flexible of the mass spectrometric techniques allowing analysis of a wide range of metabolites by modifying the chromatographic stationary and mobile phases as well as the ionization modes of the mass spectrometer. It is even possible to quantify different enantiomers should racemic mixtures of chiral metabolites be suspected. Quality control of the analysis is essential to ensure reproducible results and to remove spurious variation due to the analysis and data processing, which may include measures such as column pre-conditioning, sample order randomization, and randomized repeat analysis of quality control samples [83, 84]. To

improve the quantitation of metabolites by LC-MS, commercial standards have been developed containing predetermined mixtures of metabolites for targeted metabolomics. A number of kits, offered by Biocrates contain a range of metabolites with the most comprehensive comprising 630 metabolites, including dozens of microbiome metabolites ([www.biocrates.com](http://www.biocrates.com)). Of specific interest to this review, the kit contains a number of known stress-related components such as dopamine, cortisol, DHEAS, histamine and serotonin. Rotter et al., used one version of this kit, AbsoluteIDQ p150, to detect and identify 162 metabolites. To ensure the robustness of the quantitation, the investigators took a stringent approach to quality control that rejected highly variable metabolites ( $\text{CV} > 25\%$ ) and those with a signal intensity of less than three times baseline in more than 50% of samples. As a result, only 44 metabolites passed quality control. This limited the metabolome coverage, mainly to acylcarnitines, phosphatidylcholines and amino acids, but gives confidence in the quantitation. In addition, Rotter et al. compared three different data normalization methods (creatinine, osmolality and regression-based normalization (RBN)), which generated different, but overlapping, panels of significant metabolites. There was a high correlation between creatinine concentration and osmolality but the correlations between creatinine, osmolality and RBN normalized metabolite concentrations were low. RBN and osmolality normalization procedures showed a significant decrease in creatinine concentration. The authors do not discuss the impact of a metabolic decrease in creatinine concentration on metabolome measurement using the creatinine normalization method and chose to draw their main conclusions from the creatinine normalized data as this is the most commonly used method, allowing comparisons to be drawn with other studies.

In some applications semi-quantitative analysis may suffice depending on the study design. This is the approach taken by Chen et al. who collected one sample prior to the start of the study period and a sample at the end, with the study period being a working day. PCA was used to show which metabolites had changed from the pre-work to the post-work sample and then to identify which had changed only in air-traffic controllers (experiencing mental fatigue) but not in executives on light duties. The authors used this design to simplify quantification by assuming that a change in MS signal strength between pre-work and post-work was due to changes in metabolite concentration. Unfortunately, this does not necessarily take into account potential matrix effects on MS metabolite quantification which has the potential to introduce bias into the study. Chen et al. sought to expand coverage of the metabolome, utilizing three different chromatographic separation modes (with C18 reverse phase, HILIC and non-polar stationary phases) and two mass spectrometric ionization modes ( $\text{ESI}^+$  and  $\text{ESI}^-$ ) to detect between 11,414 and 17,590 compounds.

Urine collection is non-invasive and therefore well suited to field-based occupational studies. However, homeostatic mechanisms maintaining the internal milieu by renal excretion leads to more variable concentration of metabolites in urine. Rotter et al., collected and individually analyzed all spontaneously passed urine samples. The authors do not specify how this design was accommodated by the data analysis protocol - linear mixed effects modelling.

The three studies utilized different data analytical approaches. Chen et al., utilized OPLS-DA to discriminate between pre-work and post-work samples in air-traffic controllers. A Wilcoxon-Mann-Whitney test was used to determine the significance of the most important metabolites from the OPLS-DA loadings. Choice of classification model may be based on researcher preference or knowledge as they all perform similarly, although one study has demonstrated that OPLS-DA out-performs PCA, PLS, Support Vector Machine (SVM) or Random Forests (RF) when data sets contain subtle differences between experimental groups [85]. It is important to recognize that multivariate analyses can over-fit data, particularly when a very large number of variables (metabolites) are analyzed compared to the number of samples [86] as in this study by Chen et al. From the thousands of metabolites detected, twenty and

**Table 1.** Metabolites associated with stress related to shift work, mental fatigue and performing chores that were identified by metabolomics.

Stressor	Study Design	Analysis	Specific Metabolites Identified (Statistically significant as reported by authors)							Reference	
			Carbohydrate Metabolism	Amino Acid Metabolism	Lipid Metabolism	Vitamins/ Cofactors	Neuro-transmitters/ Hormones	Nucleic Acid Metabolism	Bile Acid Metabolism		Miscellaneous
Night Shifts	68 female nurses on shift work Longitudinal study comparing day shift with night shift	Urine LC-MS	↑	Arginine, Phenylalanine	2 Acylcarnitines Sphingomyelin C24:0						Rotter et al., 2018 [61]
Mental fatigue	45 male air traffic controllers 23 office workers with light workload as control	Urine LC-MS	↑	Creatinine	5 Acylcarnitines Phosphatidyl-choline ac C38:3		alpha-CEHC (vitamin E metabolite)				Chen et al., 2016 [62]
Occupational & Lifestyle Stress	135 male and female volunteers classified as stressed (acutely or chronic), non-stressed or borderline and by source of stressors as work, social, personal, other	Serum 1H-NMR	↑	6- Phosphogluconic acid, D-Arabitol, Sorbitol, Fructose, Threonic acid	Cysteine, Alpha aminobutyric acid, Aminoöadipic acid	2-methyl glutaric acid		N2, N2-dimethyl-guanosine, N-acetyl cytidine	Dihydroorotic acid	Chenodeoxycholic acid	Sood et al., 2013 [63]
			↓		5-hydroxytryptophan, Urocanic acid,						

fourteen metabolites were identified that contributed to the differences between pre- and post-work samples in two groups of air-traffic controllers (mental fatigue) and 35 metabolites were identified in the light-duties group. Only three metabolites were altered in both mental fatigue cohorts and not in the control condition and therefore considered to be associated with mental fatigue. This approach to reducing the number of metabolites may overcome the over-fitting issues.

Sood et al. [63] utilized OPLS-DA as a screening tool together with univariate t-test and ANOVA to determine the significance level of metabolites contributing to the separation between stressed, non-stressed and borderline stressed subjects. Significance level was set at  $p < 0.05$  and the authors do not specify corrections for multiple analyses or false discovery rate methodology. Univariate statistical analysis is often performed as a first pass data analysis in metabolomic studies. However, the problem of applying multiple statistical analyses means that statistically significant results can occur by chance when no difference is actually present in the data, leading to spurious conclusions. This is particularly problematic with the hundreds or thousands of metabolites that can be detected in a single sample. The classical probability threshold for statistical significance of 5% may not therefore be sufficiently discriminatory in this setting [87]. Using a much higher probability threshold, or applying a correction for multiple tests, can minimize erroneous identification of “significant” metabolites [86]. A number of articles have been written on methods of dealing with false positive discoveries with multiple statistical testing e.g. [88, 89, 90, 91]. Another challenge is that univariate analyses do not always identify differences between experimental groups where they exist (false negatives) because they can't identify variables that are discriminatory when combined but not when considered individually [86]. The approach of Sood et al., may therefore have led to misidentification of relevant metabolites contributing to the effect of occupational stress on the urine metabolome in this study. Sood et al. identified 41 contributory metabolites, eighteen of which had reported associations with stress or stress-related conditions. Ten were specific to the stressed cohort. In contrast, Chen et al. [62] may have missed relevant metabolites by their approach because it may have neglected combinatorial effects.

Longitudinal or time-course data sets in which subjects may act as their own control are of particular interest in clinical or field studies. Classification methods such as PCA or PLS are applicable but with a time course dimension. Analyzing the multivariate changes over time can be achieved using techniques such as multivariate analysis of variance (MANOVA) or a Repeated Measures (RM) model [90]. Rotter et al. used linear mixed effects modelling to determine the impact of shift work and chronotype on the urine metabolome, presumably controlling for urine sample collection time (although this is not specified). Fifteen of the 44 metabolites that passed QC were considered to explain a significant proportion of the shiftwork-associated variability with an acceptable false discovery rate ( $p < 0.05$ ).

### 4.3. Interpretation of metabolomic changes associated with occupational stress

The metabolites associated with occupational stress according to the original authors' criteria are listed for comparison in Table 1.

In the study of Rotter et al., female nurses on night shift exhibited a different urinary metabolome than on day shift. The effect was different depending upon the chronotype of the subject (early or late preference). In explaining the difference observed in shift work, the authors focused on nightshift induced perturbation of acylcarnitines which could indicate altered fatty acid beta-oxidation under the control of circadian rhythm regulators such as CLOCK/BMAL1. There were also alterations in the urinary levels of phospholipids and sphingolipids. Alterations in phenylalanine and arginine may indicate modified catecholamine and nitric oxide signaling, respectively. Arginine is also a precursor of creatine. Creatinine was significantly decreased following RBN or osmolality normalization procedures. Increased arginine and decreased creatinine

may indicate a decreased requirement for creatine synthesis due to lower degradation of phosphocreatine to creatinine suggesting a change in energy usage in muscles during night shift work. Night shift work is likely to be associated with disrupted circadian rhythm, sleep patterns and possibly sleep deprivation. Sleep deprivation is of general relevance in the occupational setting given the association of sleep deprivation with full-time working and an apparent societal decrease in the average number of hours spent sleeping [92, 93, 94]. Sleep deprivation of just 24 h was shown to affect the LC-MS derived metabolic profile of plasma [73], with significantly increased levels of tryptophan, serotonin, taurine, 8 acylcarnitines, 13 glycerophospholipids, and 3 sphingolipids in male volunteers. In a subsequent study in female volunteers [74] decreased histidine, glutamate, citrulline and dimethylarginine, carnitine and a number of phosphatidylcholines were observed, suggesting a sex difference in the response to sleep deprivation. While nightshift work and sleep deprivation are not an exact comparison, similar perturbations were detected in lipid metabolism with a decrease in five acylcarnitines and an increase in two other acylcarnitines reported in the female nurses on nightshifts.

The urinary metabolomic changes associated with mental fatigue were studied in air-traffic controllers [62]. In this comprehensive LC-MS/MS analysis utilizing three different chromatographic separation conditions to increase the richness of the detected metabolome, the authors reported three putative biomarkers: decreased N2, N2-dimethylguanosine, decreased N-acetylarylamine (a well-known but discontinued analgesic) and increased alpha-carboxyethyl hydrochroman, a water-soluble metabolite of vitamin E. The authors offered limited biological interpretation of the results, suggesting that N, N-dimethylguanosine belonged to the tyrosine metabolism pathway (KEGG). However, this metabolite is also associated with tRNA degradation, suggesting a down-regulation of protein synthesis in mental fatigue. Decreased N-acetylarylamine in air-traffic controllers (or increase in executives on light duties may highlight the potential for pharmaceuticals to be a factor (confounding or otherwise) in real-world studies. However, it does seem unlikely that the executives on light duties were managing headache symptoms with a discontinued analgesic. The finding that turnover of vitamin E might be increased in mental fatigue is interesting given its role as an antioxidant and the proposed role of oxidative stress in health deterioration caused by sleep loss [95]. Interestingly alpha-tocopherol (Vitamin E) was elevated in plasma in patients exhibiting exhaustion disorder [62], although the two findings appear at odds.

The study of Sood et al. is notable in that it attempts to use metabolomics to develop a diagnostic tool in itself as opposed to identifying putative biomarkers. Sood et al., used NMR-based metabolomics to evaluate the natural stress status of individuals as a result of dealing with regular chores. Scores were calculated from self-reported questionnaires of stress status (Q-Score) and from the quantification of serum metabolites identified by NMR (M-Score). There was a strong correlation between the Q-Score and M-Score among 124 subjects. Out of 187 metabolites detected, 30 contributed significantly to the M-Score and 18 were previously found to be associated with psychological stress or stress-related diseases. These included 6-phosphogluconate, amino adipate, D-arabitol, cysteine, sorbitol, D-fructose, threonate, 2-methylglutarate, chenodeoxycholate (CDCA), L-dihydroorotate. Further work is required to determine if the M-Score could be used as a biomarker to help individuals to alleviate their stress or reduce exposure to stressors, but given this approach utilizes the changes in numerous metabolites simultaneously to enumerate the score, it would appear to have significant promise for the study, management and reduction of the pleiotropic response to occupational stress.

Amongst these occupational studies (mental fatigue, shiftwork and chore-related stress), the only study to show an increase in carbohydrate metabolites was the study of Sood. This may be a function of the methodology used (NMR as opposed to MS). Notably, this was also the only study that demonstrated an alteration of cortisol. Serum cortisol was

increased consistent with stress without adaptation to a blunted cortisol response that can occur in chronic stress. CDCA has been reported to have effects on glucocorticoid metabolism by inhibiting 11-beta-hydroxysteroid dehydrogenase (11 $\beta$ -HSD) [96]. The HSD11B2 form of the enzyme oxidizes cortisol to cortisone, deactivating it in the kidneys, colon and salivary glands. Inhibition of this form could be consistent with elevated serum cortisol. In contrast, a metabolomic study of Chronic Fatigue Syndrome (CFS) reported a reduced concentration of chenodeoxycholic acid [65]. How these findings are related is not clear. Elevated dihydroorotate, a precursor in uridine monophosphate synthesis, could be the result of increased RNA synthesis (caused by increased protein synthesis) or inhibition of the enzymes utilising dihydroorotate e.g. dihydroorotate dehydrogenase (DHODH). Inhibitors of DHODH are immunosuppressive [97] and immunosuppression is a common effect of chronic stress [98].

None of the notable findings observed in occupational nightshift work [61], mental fatigue [62] or general occupational and lifestyle stress [63] corresponded between studies (Table 1).

## 5. Discussion

Stress is a multi-faceted phenomenon, characterized by different triggers, different responses and affecting the whole body. While the biological response to stress has been studied extensively over many decades [99], it has proved difficult to associate the known biochemical and physiological responses with stress in the occupational setting [23, 38]. Multivariate measures of stress, such as those produced by metabolomics, may help to address this gap. The aim of the current review was to determine the extent of the current literature on metabolomic investigations of occupational stress.

A total of thirteen clinical or field-based human metabolomic studies of stress were identified, out of which three were investigations of occupational or workplace stress. Although a limited data set, the results of the three occupational studies are consistent with the proposition that stress is diverse in its etiology and consequences and therefore in the biochemical changes that are detectable. The study of Sood et al. [63] investigated chronic stress caused by repetitive chores that may therefore conform to the JDCS model of stress [14, 15]. In contrast, the study of Chen et al. [62] into mental fatigue caused by intense mental and emotional work, investigated a relatively acute stress response (one shift) which may also conform to the JDCS model. The third study into night-shift work [61], may exemplify a mixture of demand-control stress and sleep deprivation of a relatively acute nature. None of the metabolomic changes observed were found in more than one of the studies. It is feasible that effort-reward imbalance [ERI] and organizational injustice (OI) could also be implicated in the stress responses measured [17, 21]. However, none of the studies attempted to associate metabolomic measures with specific models of occupational stress. Sood et al. diagnosed stress based on self-reported symptoms such as headache, forgetfulness, etc., while stress was not specifically measured as a construct in the other two studies. Future metabolomic studies of occupational stress might benefit from associating metabolomic changes with specific models of stress. However, as noted in the introduction, validation of metabolomics with existing psychometric models of stress may be counterproductive. Rather than validating one against the other, the aim should be to utilize all information integrated into a single model to arrive at a better understanding of the causes, chronology, responses and adaptations to stressors.

The conclusions that can be drawn from this small dataset about the metabolic signature of occupational stress are limited. As described in the results, the biochemical changes observed in each study were largely interpretable with respect to known stress responses. However, none of the changes were observed in more than one study. Clearly a larger number of studies is required, but it emphasizes the point that the changes occurring as a result of stress are complex and numerous. As an example, a number of changes specific to the nightshift study were

consistent with those observed in a metabolomic study into sleep deprivation in female volunteers [74], further supporting the proposition that occupational stress is not a single entity with predictable biochemical and physiological responses. Nightshift work and high workload may both be considered stressful because of high demand, low control and support, but are likely to have different metabolic signatures, as exemplified by the studies of Rotter et al., and Sood et al. reviewed here. Similarly, stress caused by workers' rights issues, institutionalized bullying or feelings of inadequate compensation may all be classed as OI stress but are likely to appear different at the biochemical level. Further understanding of the biochemistry of occupational stressors within the existing frameworks provided by psychological models of stress may facilitate a greater understanding of how people adapt and the consequences for long-term health.

The lack of concordance between the studies is only partly due to the different etiologies of stress in the studies. Another factor is the different methodologies used. For example, the study of Sood et al. was the only one that identified changes in carbohydrate metabolism. This is likely due to the use of NMR in this study while the other two studies used LC-MS. Unfortunately, the diversity present in biochemistry cannot currently be captured by any single analytical technique. In order to compile a complete picture of the metabolomic responses to stress it will be necessary to combine techniques (at least until a universal methodology is developed in the future). This complicates the analysis considerably. As demonstrated by Rotter et al., normalization of LC-MS data for metabolite quantification is challenging with different normalization methods producing different results. Normalizing consistently across LC-MS, NMR, GC-MS, electrochemical detection or other methods exacerbates the problem. Furthermore, the complexity of data analysis increases exponentially as the dimensionality of the data sets expand to answer such questions [100]. Technological developments are addressing this, allowing large datasets of information including hundreds or thousands of metabolites of interest to be compiled, as demonstrated by the approach of Chen et al. There is, however, a tendency to reduce these large datasets to "a change in energy metabolism" or "disturbed amino acid metabolism", with the risk of investigator-specific bias in this kind of focusing.

Another methodological difference between the studies was the choice of biofluid. Urine is relatively convenient, not requiring any special expertise to collect, and is therefore well suited to occupational studies. Urine was the matrix chosen by Rotter et al. and Chen et al. However, urine production is continuous whereas urine collection is discontinuous, complicating the interpretation of metabolite concentrations which may be affected by circadian rhythms, such as the well-known daily fluctuations in cortisol levels [101] and may mask acute or intermittent stress responses. Pooling of 24-hour collections can overcome the issue of natural rhythms but may further mask acute and intermittent stress responses. Serum, as analyzed by Sood et al., allows a greater granularity of timed samples and therefore ability to detect metabolic changes associated with acute or intermittent stress. The disadvantage of requiring trained personnel on site and interrupting work to take many, regular samples can be partially overcome by blood-spot sampling [102, 103]. This technique can be performed by the subject with minimal equipment and training to take a small thumb-prick sample [104]. It is relatively painless, although most people would prefer not to collect too many samples from themselves in a day. Such a sampling technique could be effectively combined with experience sampling methodology (ESM), which interrupts subjects going about their normal activities to determine their state in the moment [105, 106, 107]. This would provide access to metabolic signatures coincident with self-reported psychological and physical state. Blood spot analysis reduces sensitivity so is currently more applicable to MS based methods [108, 109].

The dynamics of metabolic signatures are complex and broadly affected by five different processes: (i) the synthesis and release of metabolites, including neuroendocrine signaling molecules, triggered by the

stress response (ii) distribution of metabolites around the body (iii) degradation of the metabolites and (iv) excretion. All five can be affected by the stress response itself or by confounding factors. A number of studies associating metabolomic changes with stress were excluded from the review because they were not occupational, but they offer insights into potential confounding factors. These included studies of aging [70], suboptimal health [71, 72], PTSD [67], Ayurvedic Therapy [69] and weight management [68]. Other confounding factors may also need to be controlled in occupational stress studies of real-world stressors, including smoking [110], alcohol, drug abuse and pharmaceutical usage [54], diet or dietary changes [111], exercise [112], environmental factors and infection [55, 113]. Therefore, analysis of detailed time courses, made possible with blood spot or saliva sampling, will greatly enhance interpretation of occupational stress responses. Concomitantly measuring analytes in saliva or urine and in the circulation will help to understand the dynamics of biological processes. None of the studies reviewed analyzed more than one matrix.

Another approach to aid interpretation is to develop a database of the metabolome. Studies cataloguing the human metabolome in urine, saliva and serum have been conducted. However, the metabolome will change throughout life and following large cohorts of individuals from cradle to grave will be instrumental to reliable application of metabolomics [114, 115]. The aim is to define normal but also to define the changes that occur in disease, during various life events and during stressful conditions forming a comparative database of metabolome variation. To this end a metabolic database of changes associated with disease-free ageing has been established [116] and is publicly available (<http://www.metaboage.info>). The growth in wearable health monitoring devices and the acceptance of such devices by the general public allows concomitant measurement of other physiological parameters such as heart rate variability, skin conductance, ECG, EEG or ultra-weak photon emission [117] to facilitate richer interpretation. Combination with proteomics, transcriptomics and genomics information will lead to even richer data sets [44, 118, 119]. Such a systems approach is already possible and will lead to a breakthrough in health monitoring, health improvement and disease prevention as opposed to disease detection and treatment. This would have widespread benefits beyond the occupational health sphere and is beyond the scope of occupational stress management.

Stress management in the workplace is growing in popularity [120] although evidence of success is limited [121, 122]. Monitoring the requirements for stress management and the success of interventions is necessary and objective monitoring to avoid biases of self-reporting, is attractive. A number of clinical studies have demonstrated that stress management techniques do affect the metabolome in ways that suggest stress reduction and health benefits [68, 69, 123]. Further work is required to evaluate the potential for metabolomics as an objective measure of the success of stress management in the workplace.

A significant barrier to the introduction of biological approaches to stress management monitoring is the current definition of a biomarker and more specifically the definition of an occupational biomarker. Although occupational biomarkers are commonplace in certain industries for the risk assessment of exposure to chemicals and other hazards [124], they have not been used to assess exposure to stress except in academic studies. Given the health and financial consequences of occupational stress, it is important that the definition of occupational biomarkers is extended to include stress and stress management [124]. Another potential barrier to implementation is the acceptance of bio-monitoring by employees. However, monitoring of physiological parameters is becoming commonplace with wearable technologies and is breaking down this barrier. Finally, legal and ethical considerations of employee monitoring must be considered and addressed.

Given the enormous and growing cost of occupational stress, there may come a time in the future when the stress status of employees is regularly monitored. Given the complexity of the stress response and the differences in response to different stressors, a multi-variate and objective approach will be required to individualize the titration of stress



levels and achieve optimum performance over the longer term. It is envisaged that this will most likely involve systems biology and artificial intelligence. However, the small number of existing studies demonstrate that considerable methodological development is required to expand our understanding of multivariate metabolomic changes occurring in many life settings before it can be reliably applied to monitoring and managing occupational stress.

## 6. Conclusion

The complexity of the occupational stress response merits a multivariate approach to investigation. Despite this, the literature on the application of metabolomics to studying occupational stress is limited. The studies that are available demonstrate the challenges of such an approach but also demonstrate the versatility of the approach. Metabolomic studies are technically complex but allow a non-hypothesis driven approach that could identify new biomarkers. The available studies demonstrate the ability of this approach to discriminate between different causes of stress that may not be possible with traditional stress biomarkers or psychometric approaches. The multivariate nature of metabolomics lends itself to combination with psychometrics in a way that has not yet been studied to develop new models and understanding of occupational stress.

## Declarations

### Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

### Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

### Data availability statement

No data was used for the research described in the article.

### Declaration of interests statement

The authors declare no conflict of interest.

### Additional information

No additional information is available for this paper.

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