Mini-Review

Metabolomics and Exercise: possibilities and perspectives

Renata Garbellini Duft
Alex Castro
Mara Patrícia Traina Chacon-Mikahil
Cláudia Regina Cavaglieri
Universidade Estadual de Campinas, Campinas, SP, Brazil

Abstract — Aim: This review aimed to provide an overview of the publications using metabolomics in research with physical exercises and to demonstrate how researchers have been applying this approach. **Methods:** A systematic search in the databases Web of Science, SCOPUS and PubMed was performed, with the key words: "metabolomics" OR "metabonomics" and with "metabolomics" OR "metabonomics" AND "exercise" in the title or abstract of the articles. The search period was from 2000 to 2016. Forty-four original articles were selected. The studies found were separated into four categories: metabolic responses to physical exercise, supplementation and physical exercise, sports performance, and physical exercise related to diseases. **Results:** It was possible to observe the exponential growth of the use of this approach in Sports and Health Sciences, and the four sub-fields towards which these researches involving exercise are directed, enabling a more comprehensive characterization of different metabolic profiles, as well as their study for identifying new biomarkers related to physical exercise. **Conclusions:** The possibilities of using metabolomics approach are increasing in the fields of Health Sciences, Sports, and Physical Activity. The experimental design of the study is essential to take advantage of this tool and be able to answer questions in the metabolism comprehension.

Keywords: metabolomics, metabonomics, exercise, physical exercise

Introduction

Metabolomics

Metabolomics is known as a comprehensive analysis to identify, quantify and characterize molecules with low molecular weight called metabolites. This approach is gaining more and more space in several research fields, such as in the health, exact and biological sciences, comprising the quartet of the omics sciences (genomics, transcriptomics, proteomics, and metabolomics). These platforms together can provide relevant information for understanding the so-called Systems Biology[1,2,3,4].

Metabolites are substrates and final products of cellular metabolism, that have essential roles in energy production, storage, signal transduction, apoptosis, in addition to providing an analysis of the physiological state of the organism[5,6]. Thus, the analysis of the metabolic profile by metabolomics has become a powerful tool, widely used for clinical diagnosis.

While genes and proteins are subject to epigenetic regulations, and post-transcriptional amendments, respectively, metabolites represent the direct signature of biochemical, cellular activity, and feature the same basic chemical structure regardless of species. Therefore, the metabolomic profile can be more easily correlated with phenotype, compared to genomic, transcriptomic and proteomic profiles[7].

With the use of metabolomics, biochemical analysis can be performed at various times, in the same organism, to better understand the dynamic changes in metabolism, through the changes in the concentrations of these metabolites[3]. Metabolic concentrations can vary in response to disturbances in cellular homeostasis through genetic, nutritional and pathophysiological changes, and also through physical exercise[8].

Thus, in order to better understand the various applications and classification of metabolomic approaches, some of the key concepts are defined below:

Targeted Metabolomics: an analysis directed towards a specific group of metabolites that have already been listed, focusing on one or more metabolic pathways of interest, for answering a hypothesis which has already been formulated[7].

Untargeted Metabolomics: a global analysis which aims to measure the highest number of metabolites, at the same time, in biological samples[7].

Metabolite Fingerprinting: an analysis in which the identification and quantification of metabolites are not the main purposes, but the distinction and classification of changes that have occurred in a biological sample. For this, an untargeted analysis is used[9].

Metabolite Profiling: an analysis that can be focused on a large group of metabolites, compounds related to a metabolic class or pathway, to understand their adjustment. It is a more focused kind of analysis compared to fingerprinting, as it identifies the metabolites[1,9].

Thus, these approaches have the purpose of describing in different ways the set of metabolites present in a certain metabolome[10]. Measuring the metabolome requires high-performance analytical techniques such as nuclear magnetic resonance and mass spectrometry.

Analytical Techniques used in Metabolomics

There are many combinations of techniques that can be used in metabolomics approaches, but the most widely used are nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS).

In NMR spectroscopy the absorption of electromagnetic radiation by the sample occurs. This absorption happens because of certain nuclei of the molecule, generating an NMR spectrum, which is a record of the frequencies of the absorption peaks against their intensities. The number of different orientations that a nucleus may take on when placed in a uniform magnetic field, called spin number, represents the angular momentum of a moving charge. This number can be determined by the mass and atomic number, ^{1}H and ^{13}C, being the ones most used in NMR, though ^{15}N, ^{19}F and ^{31}P also exist. Radio frequency is given in megahertz (MHz)[11].

Among the advantages of the use of NMR in metabolomics is sample preparation, which requires minimum processes, being non-destructive or invasive, and widely used for biological fluids or solid biological materials, such as tissues. The main limitations of the technique include resolution and spectral sensitivity, which can improve with the use of high-intensity magnetic fields (greater than 500MHz). Another limitation is the number of identified metabolites that is smaller than about the use of other techniques such as mass spectrometry (MS)[12].

In MS, a high mix of sensitivity and selectivity is offered. This technique provides highly specific chemical information, which is directly related to the chemical structure of the compound, such as mass precision, patterns of isotope distribution for the determination of the elemental formula, and the fragments of ions for structural elucidation or identification through the spectral combination of official compounds data. Another important factor is the high sensitivity of MS, which allows the detection and measurement from picomole to femtomole levels of primary and secondary metabolites. These advantages make MS a major analytical tool in metabolomics[13].

Within mass spectrometry, several different techniques can be applied and have different principles. Mass spectrometers with different resolutions are used in metabolomics. The choice of the specific platform for each experiment will depend on the purpose of the project, as well as on the budget for expenses with instrumentation. Mass analysis can be direct, which is not effective in the identification of metabolites. It is necessary to associate with some chromatography for separation and identification of compounds, and for generating a complex analysis of the metabolome. Among the most commonly used are gas chromatography (GC-MS), liquid chromatography (LC-MS), high-performance liquid chromatography (HPLC-MS), ultra-performance liquid chromatography (UPLC-MS) and separation through capillary electrophoresis (CE-MS)[9,13].

The spectras generated by these analytical platforms needs to be processed for data achivement.

Data Analysis

Data analysis is one of the most important parts of metabolomics since a large number of data is generated by these platforms. From the obtaining of the data, it is necessary to use bioinformatics and chemometrics, which are mathematical, statistical and graphical applications to maximize the information that can be extracted from chemical or spectral data, for the data interpretation[14].

The first step is data standardization to minimize their heteroscedasticity, due to biological variations, and variability of measurement techniques. The normalization of the samples can be made through the means, medians or an internal reference. These normalizations are commonly followed by non-linear conversions, such as logarithmic transformations, that helps to reduce the heteroscedasticity of the data enabling greater symmetry between the curves of data distribution, required for the application of linear techniques[15]. Based on this, the scaling of the data is performed, in which each variable is divided by a scale factor of the variable itself, as a measure of data dispersion (such as the standard deviation) and another of the size of the measure (for example, the mean). The most commonly reported escalation techniques are auto-scaling and Pareto scaling, which aims to adjust differences in the concentrations of metabolites, adjusting them through a relative scale factor that makes them comparable with each other[15].

Principal Component Analysis (PCA): an exploratory non-supervised analysis tool to reduce the dimensionality of the data set, increasing interpretability and minimizing the loss of information[16]. This analysis is a technique for pattern recognition and not of classification, which has as goal replacing all correlated variables by a smaller number of non-correlated variables, usually referred to as main components[17].

Partial Least Squares Discriminant Analysis (PLS-DA): a technique used to optimize the separation between samples of different groups. It is a supervised method, unlike PCA, used to show the variables (metabolites) that are most responsible for differences between the sample groups, through the calculation of principal components. The construction of a validated model is needed (cross-validation and permutations test) for it to be able to predict the classification of the variables involved in the model. Another analysis associated with PLS-DA is variable importance in projection (VIP), which shows a rank with the main metabolites participating in the segregation of the groups in the model, highlighting potential biomarkers[3,18].

Partial Least Squares Orthogonal Discriminant Analysis (OPLS-DA): a supervised technique, which facilitates interpretation and transparency compared to PLS-DA. In OPLS-DA a regression between the multivariate and variable data which contain information from only one class is calculated. The advantage compared to PLS-DA is that a single component is used as a predictor of class, while the other components describe the orthogonal variation for the first predictive component[19]. For this model to be able to predict the classification of groups, the robustness of OPLS-DA is validated using two parameters, R^2X which indicates the total variance explained in the data and Q^2 (cum) which explains the degree of separation between classes, as well as the predictability of the model. For the observation of the main metabolites in the discrimination between the groups in the model, the S-Plot graph is used, which represents the contribution of spectral variables in the segregation of groups, showing data magnitude and reliability.

There are still other secondary analyses that are typically conducted, such as Pathway Analysis, Enrichment Analysis, and Integrated Pathway Analysis to study the metabolic pathways

related to the metabolites found[20]. In addition, there are also clustering analyses such as K-means clustering, Hierarchical clustering and Self-organizing map, which aim to identify the groups in the same set of data with significant intra-group similarities and differences between groups[21].

Use of Metabolomics in Research fields

Several research fields have been using metabolomics approaches to understanding better the mechanisms of action, metabolic pathways involved in response to homeostasis disturbances, and for the identification of new biomarkers related to diseases in human beings, plants or animals[22]. Currently, the Medical Sciences have been using metabolomics for elucidation on cardiovascular diseases[23], cancer[24], obesity[25,26], diabetes[27], among others since most of the causes of these diseases are related to some metabolic dysregulation[28].

Other fields of Science have also been using the technique in studies with plants[29,30], regulation of metabolic pathways[31], environment[32], nutrition[33], and physical exercise[34,35].

Physical exercise is characterized as something which disturbs metabolic homeostasis. When carried out in different populations, intensities, protocols, and pathophysiological conditions generate a metabolic imbalance, which can be measured through the change in the concentration of metabolites by metabolomics, to try to understand the mechanisms of metabolic responses better. Thus, this review is aimed to provide an overview of the publications using metabolomics in researches with physical exercises, focusing on how researchers have been applying this new approach.

Methods

For the conducting of this review, firstly a systematic search was carried out in 3 databases: Web of Science, SCOPUS and PubMed with the keywords: "metabolomics" OR "metabonomics" in the title or abstract of the articles. The search period was from 2000 to 2016. Then, in these same databases and the same period, the following keywords were used: "metabolomics" OR "metabonomics" AND "exercise".

In the second step a search in these three databases was conducted, in the same period, using the keywords: "metabolomics" OR "metabonomics" AND "exercise" OR "physical exercise" OR "physical activity" in the title or abstract of the articles.

For the second search, only articles which met the following criteria were included in the analysis: human studies; studies with physical exercise described in detail in the methodology, studies with the use of metabolomics, studies published in English in the period from 2000 to 2016. The studies that did not fit the criteria were excluded. By the end, 44 articles had been selected (Figure 1).

Results

Publications with Metabolomics

The first search resulted in all publications with the keywords metabolomics or metabonomics found in the three databases. In Figure 2 it is possible to see the number of publications per year. From this search, an exponential increase in the number of publications was observed from 2000 to 2016, showing how much this field of research has been growing each year.

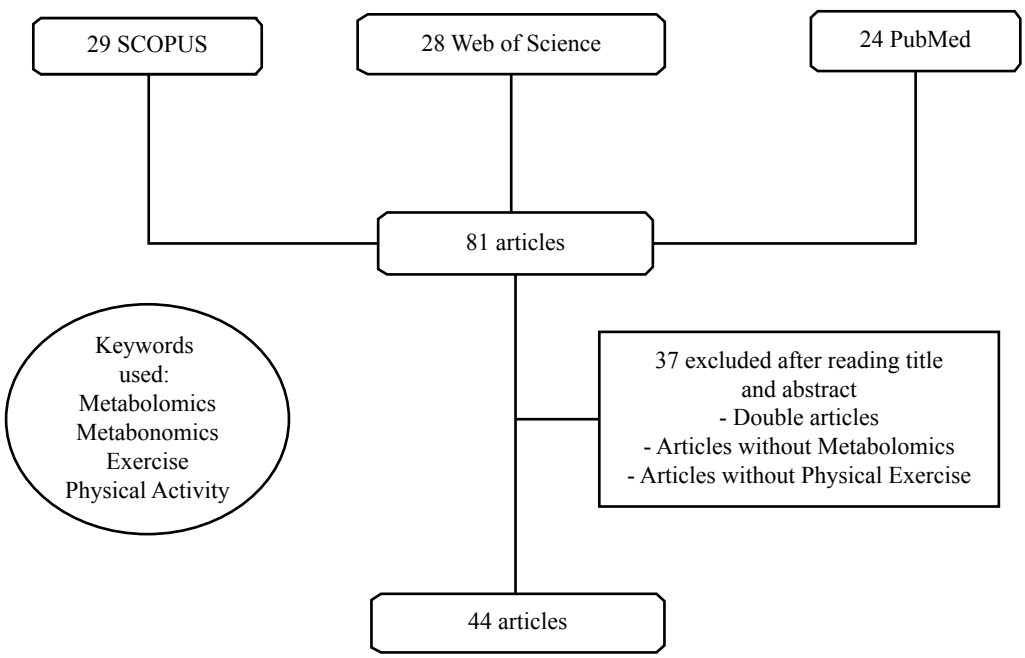

Figure 1. Flowchart of the selection of articles on metabolomics and physical exercise in databases.

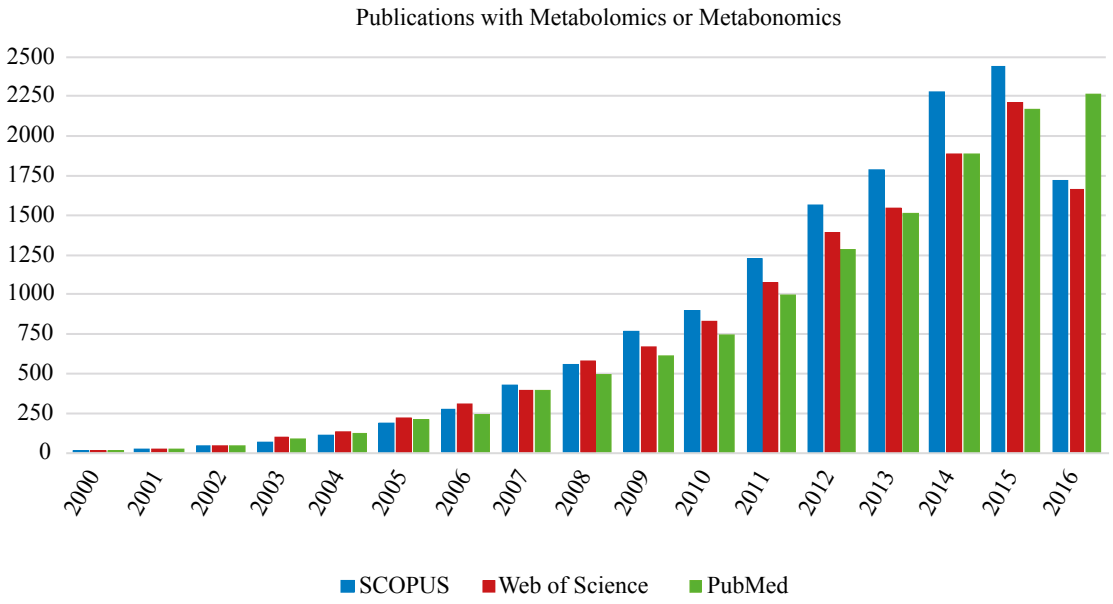

Figure 2. Number of publications per year using the key terms "metabolomics" or "metabonomics".

In Figure 3 the number of publications per year with metabolomics or metabonomics is shown, including the keyword "exercise." It is possible to see that the first publications with exercise appeared only in 2007, a period of about seven years without any related research having been observed. Comparing both pictures, it seems that there has been an increase in the number of publications each year, but looking at the total number of publications, the field of metabolomics applied to physical exercise still appears much lower in quantity.

Metabolomics and Physical Exercise

The second search conducted in the Scopus, Web of Science and PubMed databases with the keywords "metabolomics" or "metabonomics", "physical exercise" or "physical activity" resulted in a total of 81 articles. Next, the titles and abstracts of all articles were analyzed, and 37 of them were excluded, according to the flowchart shown in Figure 1, resulting in 44 selected articles. The articles were also divided into four categories: 1 – metabolic responses to exercise, 2 – supplementation and exercise, 3 – sports performance and 4 –-exercise related to diseases (Figure 4).

The complete list of selected articles with exercise and metabolomics can be seen in Table 1 which are separated by year of publication, authors, published a periodical, the methodology used, exercise protocol, type of exercise and type of intervention and category.

Discussion

This review was conducted to provide an overview of how metabolomics approach has been explored in research in the field of Sport Science and Physical Education. As well as show the ways that researchers have chosen to better understand physical exercise using this new method of analysis. To facilitate comparisons and results, we divide the articles into subtopics, which fit the most, and some of them are discussed below based on the information contained in Table 1.

Metabolic Response to Exercise

About 46% of the articles with metabolomics and physical exercise seek to investigate, using different protocols and intensities, the metabolic response to exercise in healthy young individuals (mostly). As can be seen in Table 1, 77% of the protocols presented were acute and 82% aerobic, only two of them were chronic and one acute chronic. Analyzing the exercise protocols used in those studies, 25% of them use high-intensity interval training (HIIT), and 25% continuous aerobic training protocols in an attempt to promote metabolic changes.

The study by Zafeiridis et al.[36] compared three aerobic exercise protocols of the same volume, with them being continuous, short HIIT (30 s) and long HIIT (3 min). The three protocols were performed by volunteers with a two-week interval between them, and the volumes of training were all similar. The PCA and PLS-DA graphics showed segregation between the moments before and after exercise, but there was no segregation between the training sessions. Therefore, the set of metabolic responses during aerobic exercise appear to depend more on global effort and work performed than on the specific features of each training session, because although different, they have generated similar metabolic changes. The metabolic pathways related to bioenergetics (glycolysis and the citric acid cycle) appear the ones which most changed.

In Danaher et al.[37], on the other hand, comparisons were made between two HIIT protocols with different intensities, the first of them being at 150% of the VO_2max: thirty 20s sprints and 40s rest. The second protocol had an intensity of 300% of the VO_2max and consisted of thirty 10s sprints and 50s rest. Segregation was observed between the metabolic response of

the protocols in the PLS-DA chart, and major disturbances were also found in the metabolism of fatty acids, lipids, and glycolysis in the group with 300% of VO_2max. The article by Kuehnbaum et al.[38] included a 6-week chronic HIIT intervention in overweight/obese and sedentary young women, with two sessions per week, consisting of 10 60s sprints at 90% of the VO_2max. There was a difference in metabolic response before and after the intervention; also, exercise-induced a positive adjustment in the expression of plasmatic o-acetylcarnitine, regarded as an improvement in muscle oxidative capacity.

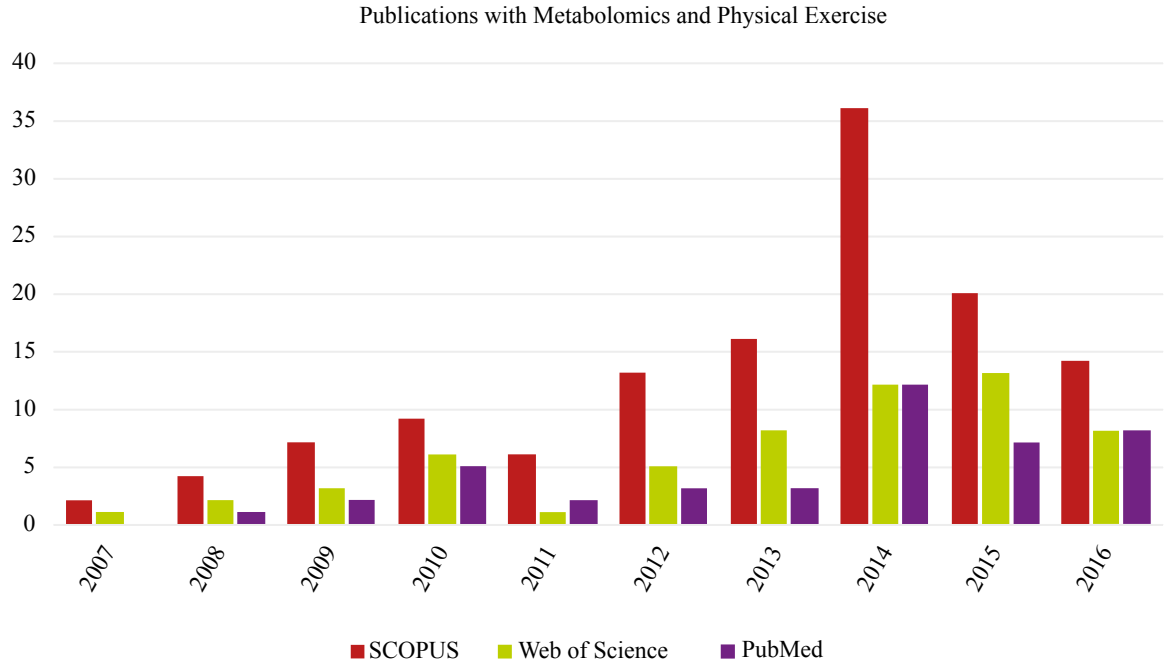

Figure 3. Number of publications per year using the key terms "metabolomics" or "metabonomics" and "exercise".

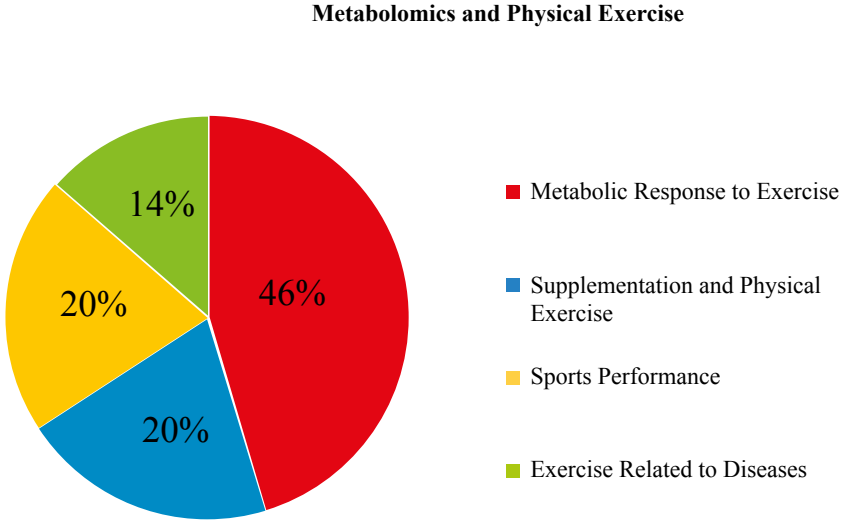

Figure 4. Percentages for the number of publications by category of classification regarding the subject of investigation.

Table 1 – Articles selected after a systematic search.

Author	Year	Title	Journal	Method	Exercise Protocol	Type of Exercise	Type of Intervention	Category
Zafeiridis et al.	2016	Global metabolic stress of isoeffort continuous and high intensity interval aerobic exercise: a comparative 1H RMN metabonomic study.	J Proteome Res	NMR	Continuous training x short (30 s) and long (3 min) HIIT in a stationary bicycle	Aerobic	Acute	1

Berton et al.	2016	Metabolic time-course response after resistance exercise: A metabolomics approach	J Sports Sci	NMR	Four sets of 10 repetitions at 70% 1RM in 45º leg press and leg extension machine	Strength	Acute	1
Danaher et al.	2016	The use of metabolomics to monitor simultaneous changes in metabolic variables following supramaximal low volume high-intensity exercise	Metabolomics	GC – MS	150% VO2max HIIT x 300% VO2max HIIT in a stationary bicycle	Aerobic	Acute	1
Muhsen et al.	2016	Metabolomic Profiling of Submaximal Exercise at a Standardised Relative Intensity in Healthy Adults	Metabolites	LC – MS	Submaximal protocol in a stationary bicycle	Aerobic	Acute	1
Hansen et al.	2015	Type 2 diabetes alters metabolic and transcriptional signatures of glucose and amino acid metabolism during exercise and recovery.	Diabetologia	HPLC – MS	60 min protocol at 50% of the Vo2max in a stationary bicycle	Aerobic	Acute	4
Glynn et al.	2015	Impact of combined resistance and aerobic exercise training on branched-chain amino acid turnover, glycine metabolism and insulin sensitivity in overweight humans	Diabetologia	GC – MS	Aerobic exercise 3-4 x/week at 65-80% of the VO2max and strength exercise in 8 different exercise equipment	Aerobic and Strength	Chronic	4
Kuehnbaum et al.	2015	Multiplexed separations for biomarker discovery in metabolomics: Elucidating adaptive responses to exercise training	Electrophoresis	MS – CE – MS	6-week HIIT, 2x/week, ten 60 s sprints at 90% of the VO2max	Aerobic	Chronic	1
Wang et al.	2015	Applying 1H RMN Spectroscopy to Detect Changes in the Urinary Metabolite Levels of Chinese Half-Pipe Snowboarders after Different Exercises	J Anal Methods Chem	NMR	Abdominal strength exercises and exercises on trampolines specific for snowboarding	Aerobic	Acute	1
Ma et al.	2015	The intervention effects of acupuncture on fatigue induced by exhaustive physical exercises: A metabolomics investigation	Evid Based Complement Alternat Med	NMR	8 km of running and 16 100 m sprints	Aerobic	Acute	3
Daskalaki et al.	2015	A Study of the Effects of Exercise on the Urinary Metabolome Using Normalisation to Individual Metabolic Output	Metabolites	LC – MS	50 min of aerobic exercise (cycling and running)	Aerobic	Acute	1
Pechlivanis et al.	2015	Monitoring the response of the human urinary metabolome to brief maximal exercise by a combination of RP-UPLC-MS and 1H RMN spectroscopy	J Proteome Res	UPLC – MS and NMR	Running (sprints) with short and long intervals between stimuli	Aerobic	Acute	1
Nieman et al.	2014	Influence of pistachios on performance and exercise-induced inflammation, oxidative stress, immune dysfunction, and metabolite shifts in cyclists: A randomized, crossover trial	PLoS ONE	UHPLC – MS and GC– MS	75 km cycling	Aerobic	Acute	3
Jacobs et al.	2014	Metabolic response to decaffeinated green tea extract during rest and moderate-intensity exercise	J Agric Food Chem	GC – MS and LC – MS	30 min stationary bicycle at 50% of the maximum power	Aerobic	Acute	2
Kuehnbaum et al.	2014	Personalized metabolomics for predicting glucose tolerance changes in sedentary women after high-intensity interval training	Sci Rep	MS – CE – MS	6-week HIIT, 18 sessions, 3x/week, ten 60 s sprints	Aerobic	Chronic	4

Nieman et al.	2014	Metabolomics approach to assessing plasma 13 – and 9-hydroxy-octadecadienoic acid and linoleic acid metabolite responses to 75-km cycling	Am J Physiol Regul Integr Comp Physiol	UHPLC – MS and GC–MS	75 km cycling	Aerobic	Acute	3
Mukherjee et al.	2014	Whole blood transcriptomics and urinary metabolomics to define adaptive biochemical pathways of high-intensity exercise in 50-60 year old masters athletes	PLoS ONE	NMR	Submaximal endurance high-intensity exercise in a stationary bicycle, 1 h in a ramp up to 90% of the VO2max	Aerobic	Acute	1
Santone et al.	2014	Saliva metabolomics by RMN for the evaluation of sport performance	J Pharm Biomed Anally	NMR	Yo-Yo Test in soccer players	Aerobic	Acute	3
Reinehr et al.	2014	Changes in the serum metabolite profile in obese children with weight loss	Eur J Nutr	HPLC – MS	Dances, games, and races	Aerobic	Chronic	4
Peake et al.	2014	Metabolic and hormonal responses to isoenergetic high-intensity interval exercise and continuous moderate-intensity exercise	Am J Physiol Endocrinol Metab	GC – MS	HIIT at 80% of the VO2max x Continuous moderate exercise at 65% of the VO2max in a stationary bicycle	Aerobic	Acute	1
Huffman et al.	2014	Metabolite signatures of exercise training in human skeletal muscle relate to mitochondrial remodeling and cardiometabolic fitness	Diabetologia	GC – MS	Aerobic and resistance exercise of low, medium and high intensity	Aerobic and Strength	Chronic	1
Sheedy et al.	2014	1H-RMN analysis of the human urinary metabolome in response to an 18-month multi-component exercise program and calcium–vitamin-D3 supplementation in older men	Appl Physiol Nutr Metab	NMR	Multi-component exercise program	Multi-component	Chronic	2
Ra et al.	2014	Metabolomics of salivary fatigue markers in soccer players after consecutive games	Appl Physiol Nutr Metab	ESI – MS	Soccer games	Soccer	Acute	3
Nieman et al.	2013	Serum metabolic signatures induced by a three-day intensified exercise period persist after 14 h of recovery in runners	J Proteome Res	UHPLC – MS and GC–MS	2.5 h of running during three days at 70% of the VO2max	Aerobic	Acute	3
Nieman et al.	2013	Influence of a Polyphenol-Enriched Protein Powder on Exercise-Induced Inflammation and Oxidative Stress in Athletes: A Randomized Trial Using a Metabolomics Approach	PLoS ONE	UHPLC – MS and GC – MS	2.5 h of running during three days at 70% of the VO2max	Aerobic	Acute	3
Yde et al.	2013	Metabonomic Response to Milk Proteins after a Single Bout of Heavy Resistance Exercise Elucidated by 1H Nuclear Magnetic Resonance Spectroscopy	Metabolites	NMR	10 x 8 reps. in a unilateral leg extension machine	Strength	Acute	2
Neal et al.	2013	Six weeks of a polarized training-intensity distribution leads to greater physiological and performance adaptations than a threshold model in trained cyclists	J Appl Physiol	NMR	Six weeks of training for cyclists	Aerobic	Chronic	3

Pechlivanis et al.	2013	1H RMN study on the short-and long-term impact of two training programs of sprint running on the metabolic fingerprint of human serum	J Proteome Res	NMR	Running sprints	Aerobic	Acute/ Chronic	1
Hodgson et al.	2013	Metabolic response to green tea extract during rest and moderate-intensity exercise	J Nutr Biochem	GC – MS and LC – MS	60 min of 50% Maximum Power in a stationary bicycle	Aerobic	Acute	2
Schubert et al.	2012	Metabolic monitoring and assessment of anaerobic threshold by means of breath biomarkers	Metabolomics	PTR – MS	Maximum effort protocol in a stationary bicycle	Aerobic	Acute	1
Li et al.	2012	Independent component analysis in non-hypothesis driven metabolomics: Improvement of pattern discovery and simplification of biological data interpretation demonstrated with plasma samples of exercising humans	J Chromatogr B Analyt Technol Biomed Life Sci	GC – MS	Two hours in a unilateral leg extension machine	Strength	Acute	1
Brugnara et al.	2012	Metabolomics approach for analyzing the effects of exercise in subjects with type 1 diabetes mellitus	PLoS ONE	NMR and GC – MS	30 min in a stationary bicycle at 80% of the VO2max	Aerobic	Acute	4
Krug et al.	2012	The dynamic range of the human metabolome revealed by challenges	FASEB Journal	Fia – MS, PTR – MS and NMR	30 min in the anaerobic threshold of each in a stationary bicycle	Aerobic	Acute	1
Netzer et al.	2011	Profiling the human response to physical exercise: A computational strategy for the identification and kinetic analysis of metabolic biomarkers	J Clin Bioinforma	MS – MS	Progressive exercise in a stationary bicycle with an increase of 25 W/min until exhaustion	Aerobic	Acute	1
Pechlivanis et al.	2010	1H RMN-based metabonomic investigation of the effect of two different exercise sessions on the metabolic fingerprint of human urine	J Proteome Res	NMR	Running sprints	Aerobic	Acute	1
Lehmann et al.	2010	Medium Chain Acylcarnitines Dominate the Metabolite Pattern in Humans under Moderate Intensity Exercise and Support Lipid Oxidation	Plos OnE	UPLC – MS	60 min continuous running at 75% of the VO2max	Aerobic	Acute	1
Bruce et al.	2010	A plasma global metabolic profiling approach applied to an exercise study monitoring the effects of glucose, galactose, and fructose drinks during post-exercise recovery	J Chromatogr B Analyt Technol Biomed Life Sci	GC – MS	HIIT: 2 min at 50% of the VO2max and 2 min at 90%. When not reaching it, it decreases to 80%, then to 70% until exhaustion.	Aerobic	Acute	2
Lee et al.	2010	Differential metabolomics for quantitative assessment of oxidative stress with strenuous exercise and nutritional intervention: Thiol-specific regulation of cellular metabolism with N-acetyl-L-cysteine pretreatment	Analytical Chemistry	CE – ESI – MS	45 min in a stationary bicycle at 75% of the VO2max and then at 90% of the VO2max until exhaustion	Aerobic	Acute	2

Enea et al.	2010	1H RMN-based metabolomics approach for exploring urinary metabolome modifications after acute and chronic physical exercise	Anal Bioanal Chem	NMR	Short: 30 s sprint at maximum speed in a stationary bicycle / Long: cycling at 75% of the VO2max until exhaustion	Aerobic	Acute	1
Miccheli et al.	2009	The influence of a sports drink on the postexercise metabolism of elite athletes as investigated by RMN-based metabolomics	J Am Coll Nutr	NMR	20 min warm-up, 1000 m at maximum intensity and 50 min in submaximal intensity in a rowing machine.	Aerobic	Acute	2
Kirwan et al.	2009	Spectroscopic correlation analysis of RMN-based metabonomics in exercise science	Anal Chim Acta	NMR	70% of the VO2max until exhaustion in a rowing machine	Aerobic	Acute	2
Chorell et al.	2009	Predictive metabolomics evaluation of nutrition-modulated metabolic stress responses in human blood serum during the early recovery phase of strenuous physical exercise	J Proteome Res	GC – MS	90 min, with nine rounds of 10 min each, with no intervals: 2 min at 40%, 8 min at 60% and 2 min at 85% of the VO2max	Aerobic	Acute	2
Yan et al.	2009	Metabolomic investigation into variation of endogenous metabolites in professional athletes subject to strength-endurance training	J Appl Physiol	GC – MS	Two weeks of technical and aerobic training for rowers	Aerobic	Chronic	3
Kuhl et al.	2008	Metabolomics as a tool to evaluate exercise-induced improvements in insulin sensitivity	Metabolomics	GC – MS	Aerobic and strength exercise during 12 weeks, 3 x/week for 50 min	Combined	Chronic	4
Pohjanen et al.	2007	A multivariate screening strategy for investigating metabolic effects of strenuous physical exercise in human serum	J Proteome Res	GC – MS	90 min, with nine rounds of 10 minutes each, with no intervals: 2 min at 40%, 8 min at 60% and 2 min at 85% of the VO2max	Aerobic	Acute	1

Note: High Intensity Interval Training (HIIT); Maximal Oxygen Uptake (VO2max); Nuclear Magnetic Resonance (NMR); Gas Chromatography-Mass Spectrometry (GC-MS); Liquid Chromatography Mass Spectrometry (LC-MS); High Performance Liquid Chromatography Mass Spectrometry (HPLC-MS); Ultra-High Performance Liquid Chromatography Mass Spectrometry (UHPLC-MS) Multi-segment Capillary Electrophoresis Mass Spectrometry (MS-CE-MS); Proton Transfer Mass Spectrometry (PTR-MS); Electrospray Ionization Mass Spectrometry (ESI-MS)

Other articles performed the comparison between HIIT and continuous moderate exercise in a stationary bicycle. In Enea et al.[39], sprinting at top speed was performed for 30 s with a 90 min break in a semi-lying position. The continuous protocol was conducted at 75% of the VO_2max until exhaustion and 90 min of recovery in a semi-lying position. There was no difference in the metabolic profile, and the study showed that the intensity of the protocol is important, because it promotes greater changes in metabolic response. In Peake et al.[40], ten 4 min sprints at 80% of the VO_2max and 2 min of recovery at 50 watts were performed. The continuous moderate protocol was conducted at 65% of the VO_2max, and the duration was given about HIIT's volume, so both protocols had the same volume. Changes occurred in the metabolism of lipids, in the citric acid cycle, and in proteins for the group that performed high-intensity interval exercise.

The studies by Pechlivanis (2010, 2013, and 2015)[41-43] assessed the acute and chronic metabolic response of the HIIT of 80 m sprints in maximum speed, but with running instead of with a stationary bicycle. The 2010 study was performed on urine samples, using three blocks 80m sprints with 10 s and 1 min intervals between them. A 20 min break was taken between the blocks and the collection held 35 min after. The 2013 study was conducted on blood samples and was a continuation of the previous survey. The same protocol of training three times a week for eight weeks was performed. There was greater segregation between the groups with 10-s interval than between the groups with a 1-minute interval in the PCA chart. In the 2015 study, three 80 m sprints with 10 min interval between the first and second and a 10 s interval between the second and the third were performed. Blood collections were performed after 1 h, 1,5 h, and 2 h. The results were presented in OPLS-DA graphs comparing the times of collection. The greatest discriminators between rest and exercise were hypoxanthine and inosine. These metabolites are part of the cycle of purines and high concentrations after exercise indicate a high rate of breakage and synthesis of ATP in muscles. Another novelty introduced by the authors changed in the metabolites of the intestinal microbiota, such as 2-hydroxy isobutyrate, TMAO, and formate.

Among the few articles that have used strength exercise protocols, Berton et al.[44] conducted a metabolic response time-course after an acute session of strength exercise in young people. A 4 series protocol with ten repetitions at 70% of 1-RM and blood collections 60 min before, before exercise, 5, 15, 30

and 60 min after exercise were conducted. Metabolites were divided between rapid response metabolites, expressed 5 min after the end of the session, intermediate response metabolites expressed 15 and 30 min after, and slow response metabolites changed only after 60 min.

Only two studies used combined training protocols (strength and aerobic). The first of these was the one by Huffman et al.[45], who investigated subjects of both genders, age between 18 and 70 years old, eutrophic or overweight and obese. The subjects were divided into several groups, with aerobic and strength exercise protocols. A combined group performed aerobic training (AT) 3 times a week at a percentage from 65 to 80 % of the VO_2max, and strength training (ST) 3 times a week, three series of 8 to 12 repetitions in exercise equipment, with exercises for the lower and upper members. The combined protocol promoted improvements in the VO_2max and insulin sensitivity, in addition to changes in metabolites from the family of acylcarnitines, which were considered markers of trainability and related to resistance and insulin sensitivity. In the study by Glynn et al.[46] overweight, sedentary, and insulin resistant subjects were invited to participate in a 6-month combined training, with 3 to 4 sessions of AT at 60-85% of the VO_2max and ST in 8 different exercise equipment. The best predictor of improvement in insulin sensitivity was considered.

In general, it should be noted that the use of exercise as a disturber of homeostasis and promoter of changes in metabolic response has increased over the years and that high-intensity interval training still seems to be the most widely used as exercise stimulus to detect possible biomarkers in metabolomics field.

Supplementation and Physical Exercise

The second category of classification is supplementation and physical exercise. It is possible to see in Table 1 that 89% of exercise interventions are acute and 78% are aerobic exercises. However, only two articles use HIIT (22%), a relatively small number compared with the number of studies seeking changes in metabolic response. Most studies used continuous moderate exercise and the intake of some kind of supplement after or during exercise.

In Kirwan et al.[47], the protocol consisted of performing an intermittent session in a stationary bicycle, followed by a low-carbohydrate meal the night before the protocol. Subjects should do ten hours fast, go back to the lab in the morning and cycle at 70 % of the VO_2max to exhaustion. Immediately after the session, and 60, 120 and 180 minutes after it, 4 g of carbohydrate were ingested. In addition, 6 mg of caffeine were ingested immediately after the exercise and 2 h after it. The authors demonstrated new information about the dynamic changes in the concentration of metabolites in plasma, in response to exercise, fatigue and nutrient supplementation during recovery, in human beings.

In the study by Jacobs et al.[48], volunteers ingested two capsules of decaffeinated green tea extract (156 ± 3 mg of green tea extract, 284 ± 6 mg of catechins, and 3 mg of caffeine) with 200 ml of water or placebo (273 ± 25 mg of cellulose) and rested for 2 h while sitting. After this period, they exercised for 30 min in a stationary bicycle at 55% of the VO_2max. These volunteers ingested, during 28 days, four capsules of the extract, two before breakfast and two before dinner. Exercise sessions took place on the first day, after seven days and after 28 days. Among the results, it was suggested that decaffeinated green tea extract increased the metabolic markers of lipid oxidation during rest after seven days, but not during exercise. After 28 days, these markers continued to increase during rest, but others also increased, suggesting an improvement in the oxidation of fatty acids in mitochondria.

The work by Yde et al.[49], is the only one that used an exclusive strength protocol. Ten series of 8 repetitions each at 80 % of 1RM and a 3 min recovery period between series were performed. After finishing, the volunteers ingested the following drinks: water, whey protein or calcium caseinate (0.3 g/kg lean body mass). Blood collections were made after 70, 220 and 370 min. The authors found differences between the groups that ingested milk proteins compared to the group that ingested water, but among the groups that ingested whey and caseinate, there was no difference in the segregation of groups.

In the study by Miccheli et al.[50] rowing athletes from the Italian Olympic team were used. The protocol was performed on a rowing ergometer, consisting of 20 min of warm-up, 1000 m in maximum speed, followed by 50 min of submaximal exercise to cause dehydration. Then the groups were rehydrated with an electrolytic beverage with green tea extract (Isotè Coop). Group B was rehydrated with oligomineral water, ingesting 25 ml every 5 min of recovery, until reaching 500 ml. A week later the groups repeated the protocol and rehydration were swapped between the groups. Blood and urine samples were collected before, immediately after the exercise and 120 min after it. There was a clear segregation in the PLS-DA models between the groups that ingested water and the electrolyte drink. Moreover, the group that ingested the drink seemed to have a better metabolic recovery than the group that ingested only water.

Based on these articles and considering the others described in Table 1, it is noted that the use of metabolomics, coupled with exercise and supplements, would help to better understanding the recovery period post-exercise, using different types of exercise protocols and supplementation on the metabolic expression analysis.

Sports Performance

In what concerns the third category of selected articles, about 20% are related to sports performance. In these studies, most volunteers are athletes of some sports modality, and the exercise protocols are related to the type of training of such modalities, such as cyclists, runners, and soccer players, among others.

The study by Yan et al.[51], used senior class rowers with over seven years experience and junior class rowers with only three years experience in China. A two-week training period before a national championship was analyzed, with a focus on speed and aerobic capacity increase. 30 hours of training were carried out, with 11 sessions a week. The collections were made before training in the first and second week and three days after

the last session and the control group did the same collections, without training. Based on the PLS-DA graph presented, it was possible to observe a large segregation between both groups with different profiles. In the second chart, it was possible to observe a different metabolic response between the basal, first and second week of training.

In Ra et al.[52] intercollegiate soccer players were analyzed to find fatigue markers in saliva. The players had three consecutive games (90 min) in 3 days. Collections were made before and after the three days. Some salivary metabolites increased after three consecutive days, and the authors suggested them as important to be studied as markers of fatigue. About the saliva markers, the study by Santone et al.[53] used the Yo-Yo test in soccer players, for performance evaluation. The athletes were divided into two groups, those who had good results in the trial and those who had bad results. The collections were made before and after the three days of games. There was no difference in the metabolic profile of the athletes who responded well and badly to the test, and there were changes in some metabolites after the test. The authors claim that saliva can be a promising biofluid for the measurement of performance in athletes.

Another example is the study by Nieman et al.[54] which was carried out with cyclists who participate in road tests. The athletes performed a 75 km protocol in their competition bikes in CompuTrainer Pro model 8001 at the laboratory. The course was hilly and of medium difficulty. Blood samples were taken before the exercise while fasting, immediately after it, 1,5 h and 21 h after it. It was possible to observe the metabolic changes that occurred from the moment before to the time after the protocol, as well as for 1,5 h and 21 h after it. The biggest changes took place in the metabolites of the metabolism of lipids and carnitine.

Unlike other studies with physical exercise, those related to sports performance did not use pre-made training protocols, but the athletes' training protocols, for the metabolic research. Diet control was far more rigorous, and the stimuli, both under load, as in duration, were much greater due to the physiological condition of these athletes.

Exercise Related to Diseases

The last category of classification of articles is the one, which relates physical exercise to diseases. These studies used therapeutic exercise supporter of diseases, such as insulin resistance, diabetes, and obesity, as noted in Table 1. Thus, the types of intervention are mostly chronic, unlike the others, which have been presented in this review. The kind of exercise is not only aerobic, but the combination of strength and aerobic training, or combined training.

In the study by Kuhl et al.[55], a 50-minute protocol was performed, three times a week for 12 weeks, combining aerobic and strength exercises in the same session by healthy people and people with type 2 diabetes. In the OPLS-DA models, it was possible to note a segregation between healthy individuals and the control group, in addition to a large segregation between trained and the control group, healthy and with diabetes, showing that exercise was a great way to improve insulin sensitivity and that metabolomics may be used as a diagnostic tool.

The study by Kuehnbaum et al. 2014[56], on the other hand, recruited overweight and obese sedentary women to perform a 6-week HIIT protocol, three times a week, for a total of 18 sessions. Each session was composed of ten 60 s sprints at 90 % of the HRmax and 1-minute interval between sprints. The authors suggested that the use of metabolomics, to find biomarkers which act as predictors of changes in glucose tolerance, in sedentary women, after interval training, is an efficient tool.

In Reinehr et al.[57] a lifestyle intervention was performed in overweight and obese children, for weight loss. The intervention consisted of nutritional, psychological, medical guidance and physical exercises during one year. The physical exercises were mostly aerobic and included different sorts of games since the target population was overweight and obese children. The results showed that some metabolites affected by obesity had their effect reversed due to weight loss, but some showed irreversible changes and remained the same after the changes in lifestyle.

By looking at these examples of studies, it may be noted that the focus of these studies is not to show a metabolic response to exercise, but to use exercise as a metabolic change and metabolic homeostasis control tool, associated with morbidities such as DM2, obesity, and others.

Final Considerations and Future Prospects

With this review, it was possible to show what kinds of research are being conducted with metabolomics in the Health Sciences and Physical Activity fields. It was possible to note the exponential increase of the use of metabolomics in researches over the last 16 years. The earliest records of the use of metabolomics in research with physical exercise were around 2007, and have been growing consistently, albeit in a slight fraction compared with the total number of metabolomics studies.

This review showed the four sub-fields towards which these researches involving exercise are directed: metabolic responses of physical exercise in different protocols and intensities. Physical exercise combined with supplementation to understand the organism's metabolic changes and the recovery process. Sports performance, with analysis of metabolic changes in athletes in their training procedures, and exercise related to diseases, using it as a form of non-pharmacological therapy in the treatment of comorbidities, and as an attenuator of metabolic disturbances caused by diseases in the organism's homeostasis.

With this, we can expect that the use of the metabolomics on sports science will continue growing. The current studies still have an exploratory character, in the understanding of metabolism as a separated compartment. However, in the near future, it is believed that the focus of research will be directed towards a better understanding of particular groups of metabolites that have been exploited, and are expressed in certain conditions (protocols, intensities), to be able to find metabolites which act as predictors of changes in metabolic profiles and integrated systems. They should be measured quickly, or even that metabolites

are expressed based on certain exercise protocols, and that they may be ingested in an exogenous manner, to improve the performance of a person in terms of aerobic capacity, strength, lean mass gain, fat mass loss, among many other beneficial effects of physical exercise in relation to the treatment of comorbidities.

The paths to be followed and the possibilities of using this approach are numerous. The techniques, methods of data analysis and interpretation are also numerous. It is up to the researchers to find the best experimental design as possible to take advantage of this tool and to be able to answer their questions, making Science further progress in the understanding of metabolism.

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Corresponding author

Cláudia Regina Cavaglieri
Adress: Av. Érico Veríssimo, 701, Cidade Universitária "Zeferino Vaz" Barão Geraldo, Campinas. SP, Brazil.
Email:cavaglieri@fef.unicamp.br

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