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Basal profile and correlation between biomarkers and other physiological parameters in mixed martial arts fighters after 72 hours of total rest

Perfil basal e correlação entre biomarcadores e outros parâmetros fisiológicos em combatentes de artes marciais mistas após 72 horas de descanso total

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ABSTRACT
The effects of physical exercise, whether for performance or health, on metabolism and the immune system have long intrigued researchers and professionals. The main objective was to investigate and present the baseline values, after a total rest of 72 hours, of blood and urine biomarkers and other

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physiological variables in high-level MMA athletes, drawing a correlation between these data with the heat map strategy. 16 male individuals participated in the study. All individuals were on absolute rest for 72 hours. Blood and urine have collected from participants at rest and fasting. In these fluids, eosinophils, neutrophils, lymphocytes, monocytes, cortisol, lactate, ketone, pH, sodium, potassium, chloride, calcium, CK-MB, D-dimer, Cystatin C, urate, phosphatase alkaline, and microalbuminuria have been measured. The Handgrip Strength has obtained with a Jamar® handgrip dynamometer. The highest positive and negative correlation coefficients (CC), as well as their P values, were presented between body mass and body mass index (CC = 0.946), between body mass and blood lactate (0.857), body mass and lactate (0.788), height and body mass (0.788), calcium and sodium (0.765), height and lactate (0.742), calcium and alkaline phosphatase (0.676), height and urate (0.641), CK-MB and alkaline phosphatase (0.637) D-dimer and alkaline phosphatase (0.618), in addition to negative correlations between lymphocytes and neutrophils (-0.894), cystatin C and D-dimer (-0.788), cortisol and sodium (-0.626), cortisol and calcium (-0.595), chloride and lymphocytes (-0.587), chloride and microalbuminuria (-0.587) and chloride and handgrip strength (-0.580). Physical Education and Sports Science are increasingly entering the field of public health, whether in epidemiological studies or health profiles, as an important agent in the promotion, protection, and recovery of the world population's health. Studies with reference values that seek a correlation between biomarkers and other variables are among the most read and cited when preparing a project in sports medicine. The present study reaffirms correlations already well known by science and proposes future research perspectives aiming at the performance of athletes and exercise practitioners and their longevity with quality of life.

Keywords: sportomics, sports medicine, physical education, exercise.

RESUMO

Os efeitos do exercício físico, seja pelo desempenho ou pela saúde, sobre o metabolismo e o sistema imunológico há muito intrigam pesquisadores e profissionais. O principal objetivo era investigar e apresentar os valores de base, após um descanso total de 72 horas, dos biomarcadores de sangue e urina e outras variáveis fisiológicas em atletas de MMA de alto nível, desenhando uma correlação entre estes dados com a estratégia do mapa térmico. 16 indivíduos do sexo masculino participaram do estudo. Todos os indivíduos estiveram em repouso absoluto por 72 horas. O sangue e a urina foram coletados dos participantes em repouso e jejum. Nestes fluidos, foram medidos eosinófilos, neutrófilos, linfócitos, monócitos, cortisol, lactato, cetona, pH, sódio, potássio, cloreto, cálcio, CK-MB, D-dímero, cistatina C, urato, fosfatase alcalina e microalbuminúria. A resistência à aderência foi obtida com um dinamômetro de aderência Jamar®. Os maiores coeficientes de correlação positiva e negativa (CC), assim como seus valores de P, foram apresentados entre massa corporal e índice de massa corporal (CC = 0,946), entre massa corporal e lactato de sangue (0,857), massa corporal e lactato (0,788), altura e massa corporal (0,788), cálcio e sódio (0,765), altura e lactato (0,742), cálcio e fosfatase alcalina

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(0,676), altura e urato (0. 641), CK-MB e fosfatase alcalina (0,637), D-dímero e fosfatase alcalina (0,618), além de correlações negativas entre linfócitos e neutrófilos (-0,894), cistatina C e D-dímero (-0. 788), cortisol e sódio (-0,626), cortisol e cálcio (-0,595), cloreto e linfócitos (-0,587), cloreto e microalbuminúria (-0,587) e resistência ao cloreto e ao punho (-0,580). A Educação Física e a Ciência do Esporte estão entrando cada vez mais no campo da saúde pública, seja em estudos epidemiológicos ou perfis de saúde, como um agente importante na promoção, proteção e recuperação da saúde da população mundial. Estudos com valores de referência que buscam uma correlação entre biomarcadores e outras variáveis estão entre os mais lidos e citados na preparação de um projeto em medicina esportiva. O presente estudo reafirma correlações já bem conhecidas pela ciência e propõe perspectivas futuras de pesquisa visando o desempenho dos atletas e praticantes de exercício físico e sua longevidade com a qualidade de vida.

Palavras-chave: sportomics, medicina esportiva, educação física, exercício.

1 INTRODUCTION

The effects of physical exercise, whether for performance or health, on metabolism and the immune system have long intrigued researchers and professionals 1-3(Labra et al. 2015; Moghetti et al. 2016; Scheffer & Latini, 2020).

The new field of science, called sportomics, seeks to reproduce the real conditions of different sports and training methods, aiming to understand their impact on immunometabolism and find the most suitable biomarkers for this monitoring 4-6 (Gonçalves et al. 2012; Lopes et al. 2019; Verli et al. 2021).

Using reference values provided by clinical laboratories to assess athletes and exercisers is not an adequate practice since each sport modality induces chronic metabolic and immune adaptations, which alter the baseline values in these individuals. Therefore, knowing the reference values for each modality and the correlation between these variables is of paramount importance for Sports Medicine.

The main objective was to investigate and present the baseline values, after a total rest of 72 hours, of blood and urine biomarkers and other physiological variables in high-level Mixed Martial Arts (MMA) athletes, drawing a correlation between these data with the heat map strategy.

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2 MATERIAL & METHODS

2.1 PARTICIPANTS

- Initially, 120 individuals were identified as eligible to participate in the study, which has been examined to confirm this eligibility; of these, 32 have excluded for having used anabolic substances in the last two years, leaving 88 individuals; of these, 12 had osteoarticular lesions in the same period, leaving 76; of these, 26 used thermogenic in the last six months, leaving 50 individuals; of these, 24 did not follow the rest protocols and were excluded, of these ten were not fasting at the time of collection, leaving the final sample of 16 individuals.

- 16 male individuals participated in the study.

- 16 high-level MMA athletes were at rest for 72 hours.

2.2 ETHICS STATEMENT

The investigation met the requirement for research in human beings (National Health Council, 2012) approved by the Ethics and Research Committee, number 2,230,073 of the Federal University of Mato Grosso (UFMT), registered at clinicaltrials.gov (NCT 03522883). All volunteers gave written consent after being informed about the nature and procedures of the study.

2.3 DESIGN EXPERIMENTAL

This is an observational and cross-sectional cohort study, widely used in epidemiological assessments, of the subgroup of studies in special populations.

All individuals were on absolute rest for 72 hours, had no previous injury or health problems, were not using illicit or thermogenic ergogenic resources, and were fasting on the day of the experiment.

2.4 DATA COLLECTION

Blood and urine have collected from participants at rest and fasting.

In these fluids, eosinophils, neutrophils, lymphocytes, monocytes, cortisol, lactate, ketone, pH, sodium, potassium, chloride, calcium, CK-MB, D-dimer, Cystatin C, urate, phosphatase alkaline, and microalbuminuria have been

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measured. For these analyses, the following equipment has used: Piccolo® General Chemistry 13 rotor (Abaxis, CA, USA), I chroma alpha® Biosys (Boditech Med Inc., Gangwon-do, Korea), spectrophotometers, optical microscopes, manual hematology counters, digital Bain Marie, centrifuges, and panoptic hematological dye.

The Handgrip Strength has obtained with a Jamar® handgrip dynamometer, with a 0.5 Kg graduation and a maximum capacity of 100 KgF.

A digital scale (Aqua plenum® with a capacity of 180 kg and a graduation of 100 grams has used. 2.1 meters) to measure the body mass. An Omron® blood pressure device model HEN 6124 has been used, and heart rate has been measured with a Polar® RS 800 CX digital device.

2.5 STATISTICAL ANALYSIS

We apply Spearman's test between all data, so we make a heat map with results. The values ranged from deep red to 1 to deep blue to -1, passing through white at zero in this heat map.

2.6 RELATIONSHIP BETWEEN COLORS AND CORRELATION VALUES IN THE HEAT MAP

The darker blue color corresponds to the negative correlation (-1), passing through lighter blue and green tones from dark to white between -1 and 0. For positive correlations, from value 0 passing through yellow tones from light to dark and then shades of light to dark red between 0 and +1. In short, greater negative correlation in dark blue and a greater positive correlation in dark red.

3 RESULTS

Table 1 presents the characteristics of the sample so that other researchers, professionals, and physicians can compare future data. Thus, the average age of the sample was 30 years (±5.06 SDM), body mass of 80 kg (± 13.8 SDM), the height of 1.8 meters (± 0.05 SDM), and body mass index of 25.6 kg/m2 (± 3.56 SDM).

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Table 2 shows the mean values, medians, error, and standard deviation of the means for the cellular and acellular components of blood and urine, in addition to the handgrip strength as data for the general condition of the individuals, these data being essential to be used as reference values of these variables at rest for high-level MMA athletes, enabling professionals to evaluate their athletes and patients, controlling and observing possible deviations from this pattern.

To construct figure 1, we initially sought the correlation between all variables used in this study through Spearman's Rank Correlation test and the values found, ranging from the highest negative correlation (-1) to the highest positive correlation (+1). From Spearman's Rank Order Correlation values, a heat map has made the representation in dark blue for the greatest negative correlation (-1) to the greatest positive correlation (+1), represented in dark red.

The highest positive and negative correlation coefficients (CC), as well as their P values, were presented in table 3 in descending order, where it was possible to observe strong positive correlations between body mass and body mass index (CC = 0.946), between body mass and blood lactate concentration (0.857), body mass and lactate index (0.788), height and body mass (0.788), calcium and sodium (0.765), height and lactate (0.742), calcium and alkaline phosphatase (0.676), height and urate (0.641), CK-MB and alkaline phosphatase (0.637) D-dimer and alkaline phosphatase (0.618), in addition to negative correlations between lymphocytes and neutrophils (-0.894), cystatin C and D-dimer (-0.788), cortisol and sodium (-0.626), cortisol and calcium (-0.595), chloride and lymphocytes (-0.587), chloride and microalbuminuria (-0.587) and chloride and handgrip strength (-0.580).

These main correlations presented in Table 3 have been used to make the heat map (Figure 2) and bar graphs (Figure 3) to facilitate this data visualization.

4 DISCUSSION

The positive correlations between body mass, height, and body mass index were already expected and widely known in the literature and professionals, being widely used in population and public health studies 7-10(Zhu et al. 2019;

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Child et al. 2019; Durá-Travé et al. 2020; Gent et al. 2020), increasing the importance of reference values and correlation studies.

The correlations between the variables mentioned above and the plasma lactate concentration have also been expected since lactate is formed in the muscle, being a proton acceptor and the most important mechanism for regulating pH in this environment. So, it has been expected that the greater the muscle mass of the individual, the greater the production of this compound 4,11,12(Gonçalves et al., 2012; Andeva-Andany; Vasconcelos et al., 2020). One more possible action of lactate has recently been proposed to modulate the anion gap. However, some researchers confuse this relationship by trying to attribute acidosis to lactate 13(Weemaes et al., 2019) when it has already known that lactate is a consequence of acidosis and not the cause of this phenomenon 14(Robergs et al. 2004) and that not even acidosis conditions cause muscle fatigue, but that intracellular acidosis increases muscle excitability during contraction 15(Pedersen et al. 2004).

Many researchers confuse lactate with lactic acid, but it is known that due to the pH of muscle, the Henderson Hasselbach equation showed that due to the pKa of lactic acid, it is impossible to produce this acid in human muscle 14,16(Robergs et al. 2004; Walburg, 1922), and even when this acid has added to the muscle in experiments, it protects against fatigue 17(de Paoli et al. 2007). And the theory that the cytoplasmic control mechanism of glycolysis is affected by the presence of oxygen during exercise has been broken, as the glycolytic flow is independent of the state of oxygenation and metabolic feedback but proportional to muscle activation 18(Conley et al. 1998). However, lately, this biomarker has been associated with modulation of the immune system, receiving special attention in this field of research and leaving the field of fatigue and performance a little 19-21(Pucino et al. 2017; Brooks, 2020; Certo et al. 2021).

The correlations between sodium and calcium, cortisol and calcium, and between cortisol and sodium are noteworthy because the concentration of these electrolytes has associated with greater chances of developing neurodegenerative disorders in the future 22(Rajput et al. 2021), even more so

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when these electrolytes show correlation with cortisol, which represents an important biomarker of stress and catabolism 23(Fogelman & Canli, 2018), an important modulator of the immune system 24(Buford & Willooughby, 2008) and also a biomarker associated with the development of neurodegenerative diseases such as the electrolytes mentioned above 25(Orti et al. 2017). And more and more, the preservation of the future health of athletes comes under discussion.

The negative correlation between chloride and lymphocytes, microalbuminuria, and handgrip strength again brings out the modulation of this ion in metabolism, with a strong influence on the immune system, preservation of nutrients, control of activation of membrane pumps in various tissues, osmoregulation, glomerular filtration, with impact on handgrip strength, which was the functionality variable of choice 26-29(Zadunaisky, 1996; Gerencser & Zhang, 2003; Juel & Massey, 2007; Ajouaoi et al. 2020).

The correlation between plasma urate and athletes' height is already well known, since, during physical exercise, the myokinase enzyme promotes the resynthesis of Adenosine Triphosphate (ATP) from two Adenosine Diphosphates (ADPs), leaving an AMP, which will suffer deamination by the AMP deaminase enzyme, resulting in two metabolites, ammonia which will be eliminated in the form of urea formed by the liver and excreted by the kidneys, and Inosine Monophosphate (IMP) which from the purine pathway will form urate, also excreted by the renal system 30-32(Barkulis & Lehninger, 1951; Kielley & Kielley, 1951; Savabi et al. 1986).

The d-Dimer biomarker, traditionally studied by cardiologists and angiologists as a biomarker of venous thromboembolism, reappeared during the COVID-19 pandemic as it is the most acute marker of the chances of progression to the serious state of the disease and is currently a source of research for different forms of stress and an important future field for the field of Physical Education and Sport 33(Neto et al. 2021).

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5 CONCLUSION

Physical Education and Sports Science are increasingly entering the field of public health, whether in epidemiological studies or health profiles, as an important agent in the promotion, protection, and recovery of the world population's health.

Studies with reference values that seek a correlation between biomarkers and other variables are among the most read and cited when preparing a project in sports medicine.

The present study reaffirms correlations already well known by science and proposes future research perspectives aiming at the performance of athletes and exercise practitioners and their longevity with quality of life.

PROTOCOL LIMITATIONS

As MMA represents an individual sport, where many teams have only one high-level athlete, this was the largest possible sample, meeting all inclusion and exclusion criteria.

CONFLICT OF INTERESTS

We declare that there are no conflicts of interest in this study.

AUTHOR CONTRIBUTIONS

LCOG, AMMN, and CMBA conceived and designed the study. LCOG, AMMN, and MVAV collected the data. LCOG, AMMN, MVAV, and CMBA analyzed the data and wrote the manuscript. All authors read and provided critical feedback on the manuscript before approval.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

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All procedures performed in studies involving human participants followed the ethical standards of Leeds Beckett University School of Sport research ethics committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

SIGNIFICANCE

- Physicians, exercise physiologists and other professionals who deal with athletes and exercisers need to know the baseline values of key biomarkers in order to maintain public health.
- Based on this knowledge, these professionals better plan training and recovery, avoiding physical, functional problems and even more severe damage such as rhabdomyolysis and renal failure.
- Physical exercise can be an important ally for health, but the stimulus must be adequate, since when weak it does not generate changes and when excessive it causes damage.
- Sports studies aim to understand the immunometabolic alterations induced by different sports and propose adequate recovery time, aiming at the return of physiological variables to baseline values.

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ANEXOS

Table 1. Subject's characteristics.

	Mean	Median	SEM	SDM
Age (years)	30.4	29.5	1.26	5.06
Body mass (Kg)	80.7	78.5	3.46	13.8
Height (m)	1.8	1.78	0.01	0.05
Body Mass Index (Kg/m^2)	25.6	25.5	0.88	3.53

Table 2. Subject's rest muscle status, cells, and biomarkers.

	Mean	Median	SEM	SDM
Neutrophils (%)	53.8	57.0	2.75	10.65
Lymphocytes (%)	36.0	33.0	2.48	9.59
Eosinophils (%)	3.8	4.0	0.57	2.21
Monocytes (%)	5.9	6.0	0.70	2.71
Microalbuminuria (mg/L)	9.97	8.37	1.268	5.073
Handgrip Strength (Kgf)	45.4	46.5	2.06	8.23
Ketone (mg/L)	0.21	0.20	0.02	0.09
pH	7.33	7.34	0.006	0.024
Sodium (mmol/L)	138.4	139.0	0.58	2.26
Potassium (mmol/L)	3.9	3.9	0.07	0.29
Chloride (mmol/L)	102.8	102.0	0.63	2.43
Calcium (mmol/L)	1.24	1.25	0.014	0.053
Lactate (mmol/L)	1.24	1.08	0.232	0.927
CK-MB (ng/L)	8.86	6.20	1.77	6.86
Cortisol (nmol/L)	448.2	444.1	19.65	76.1
D-dimer (ng/mL)	110.1	118.6	10.69	41.41
Cystatin C (mg/L)	5.74	6.5	0.48	1.86
Urate (umol/L)	282.8	269.0	19.39	77.56
Phosphatase alkaline (U/L)	68.2	66.0	5.57	22.27

Table 3. Main Spearman test correlations.

	Type	Correlation Coefficient	P-Value
Body mass index and body mass	Positive	0.946	0.0000002
Body mass and lactate	Positive	0.857	0.0000002
Body mass index and lactate	Positive	0.788	0.0000002
Height and body mass	Positive	0.788	0.0000002
Calcium and sodium	Positive	0.765	0.000391
Height and lactate	Positive	0.742	0.000644
Calcium and phosphatase alkaline	Positive	0.676	0.00539
Height and urate	Positive	0.641	0.00720
CK-MB and phosphatase alkaline	Positive	0.637	0.0104
D-dimer and phosphatase alkaline	Positive	0.618	0.0136
Lymphocytes and neutrophils	Negative	-0.894	0.0000002
Cystatin C and D-dimer	Negative	-0.788	0.0000002
Cortisol and sodium	Negative	-0.626	0.0158
Cortisol and calcium	Negative	-0.595	0.0235
Chloride and lymphocytes	Negative	-0.587	0.0262
Chloride and microalbuminuria	Negative	-0.587	0.0211
Chloride and handgrip strength	Negative	-0.580	0.0231

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Figure 1. Heat map about Spearman's correlation between the variables.

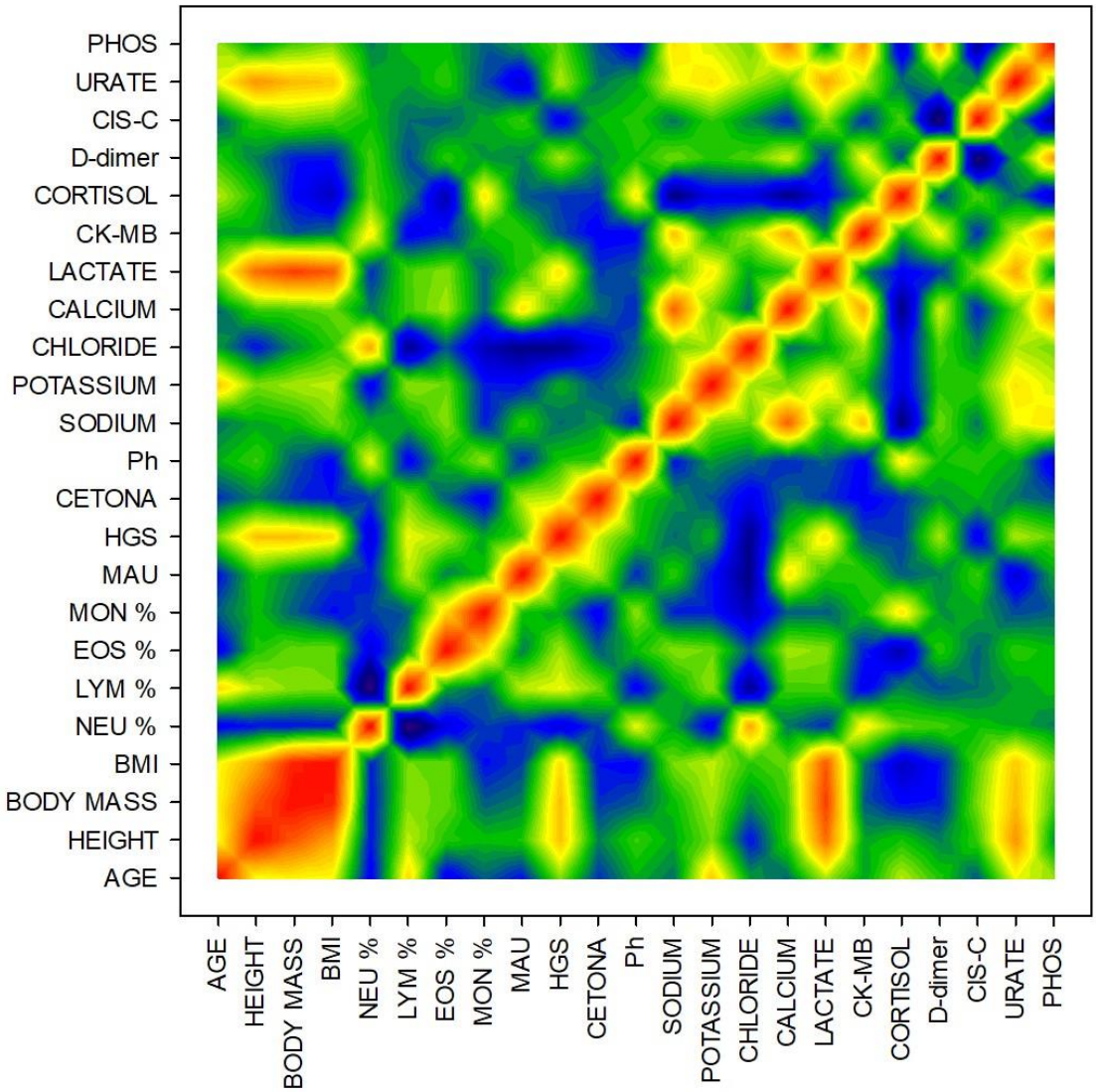

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Figure 2. Heat map about main Spearman's correlation values (based on table 3).

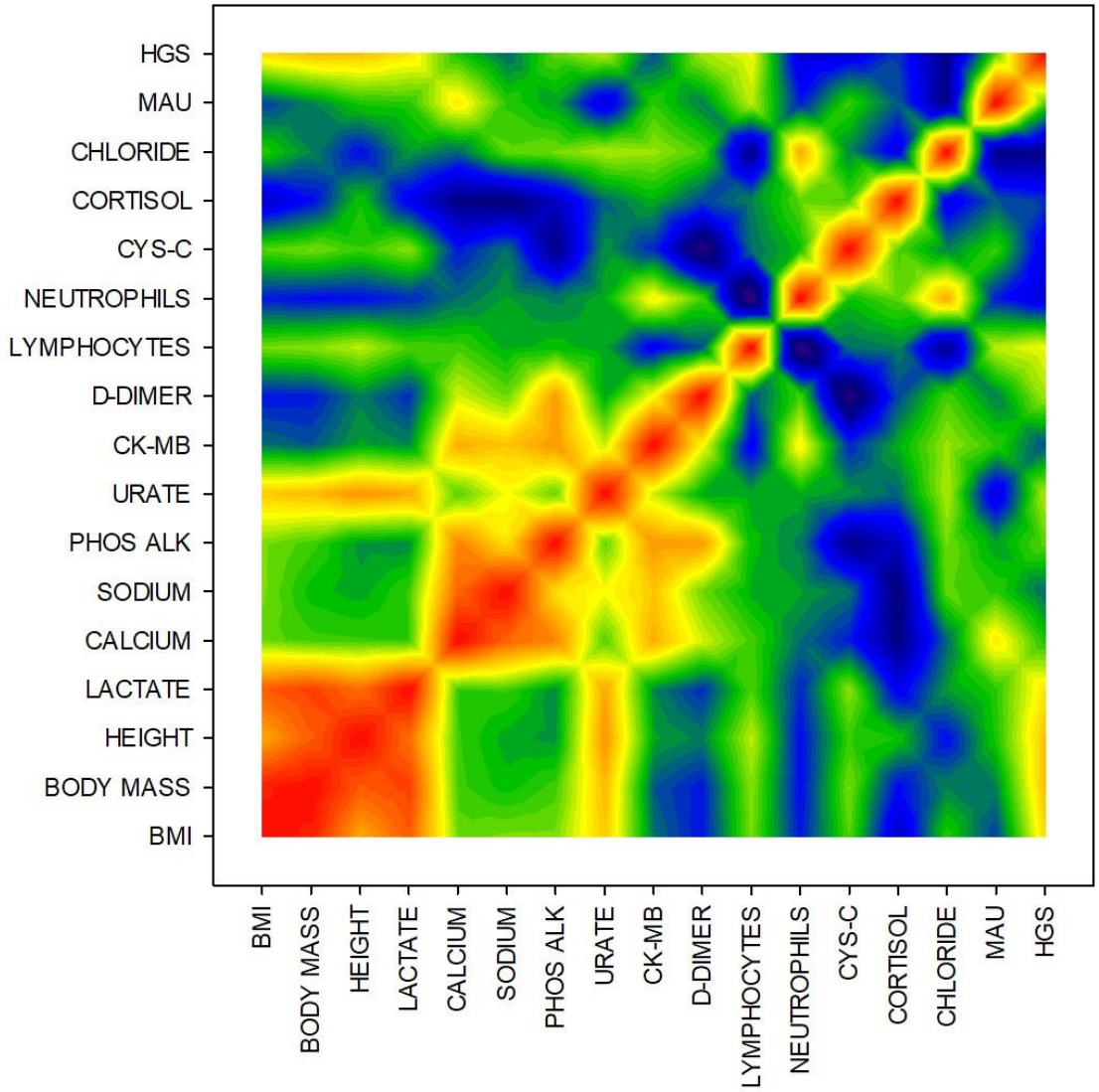

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Figure 3. Representation of correlation coefficients and P values (based on table 3).