Method for the determination of the amino acids for myocardial infarction and diabetes type 2.

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Introduction

The aim of the study was to develop and validate a method for the determination of the amino acids ***homocysteine, valine, methionine, isoleucine, leucine, tyrosine and phenylalanine*** of the amino acids in human blood plasma. Cardiovascular diseases are the number one cause of mortality worldwide. Studies over the past 10 to 20 years have shown that elevated homocysteine is a marker of risk of cardiovascular disease. Homocysteine is an amino acid measured in plasma and the normal levels are in the range 5 - 15 nmol / ml blood plasma.

Sample Preparation and Analysis

500 µl blood plasma and 100 µl 4% dithiothreitol solution were mixed in a 1.5 mL tube and incubated by 40° for 30 min. Bound forms of homocysteine in the sample are reduced in form of free homocysteine by the use of 4% dithiothreitol solution. After the incubation 150 µL of precipitation solution were added and deposit in the refrigerator for 20 min for the protein precipitation. Then 500 µL of sample dilution buffer (including internal standard norleucin, 100 nmol/mL) were added and mixed. The supernatant was filtered with a membraSpin by centrifugation at 14000 rpm for five minutes. The particle free solution was used for the injection.

The samples were analyzed by the Amino Acid Analyzer ARACUS, manufactured and distributed by membraPure GmbH worldwide. ARACUS is using the classic routine analysis of amino acids by post-column derivatization with ninhydrin and the detection at 440 nm and 570 nm.

Figure 1: Amino Acid Analyzer ARACUS

Results & Discussion

Table 1 shows the results from patients without and patients with myocardial infarction. The concentration of homocysteine increases by patients with myocardial infarction.

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Table 2 shows the results from patients without and patients with diabetes type 2. The concentration of the AA Leucine and Tyrosine increases and the level of Phenylalanine decreases. Homocysteine increases, so the patients with diabetes type 2 have also a high risk of developing cardiovascular diseases.

Table 1: Amino acid concentration of valine, homocysteine, methionine, allo-isoleucine, isoleucine, leucine, tyrosine, and phenylalanine in nmol/mL plasma of patients without myocardial infarction.

Amino Acid	Mean $\bar{x}$	SD
Val	197.6	± 28.7
H-Cysteine	10.7	± 4.0
Met	22.9	± 5.2
Ile	66.3	± 9.4
Leu	124.1	± 25.2
Tyr	65.5	± 5.4
Phe	101.4	± 21.8

Table 2: Amino acid concentration of valine, homocysteine, methionine, allo-isoleucine, isoleucine, leucine, tyrosine, and phenylalanine in nmol/mL plasma of patients with myocardial infarction.

Amino Acid	Mean $\bar{x}$	SD
Val	250.4	± 48.5
H-Cysteine	29.7	± 4.2
Met	26.7	± 7.3
Ile	88.4	± 12.4
Leu	159.1	± 23.7
Tyr	83.5	± 21.9
Phe	102.6	± 27.4

Table 3: Amino acid concentration of valine, homocysteine, methionine, allo-isoleucine, isoleucine, leucine, tyrosine, and phenylalanine in nmol/mL plasma of patients without Diabetes Type 2.

Amino Acid	Mean $\bar{x}$	SD
Val	197.6	± 28.7
H-Cysteine	10.7	± 4.0
Met	22.9	± 5.2
Ile	66.3	± 9.4
Leu	124.1	± 25.2
Tyr	65.5	± 5.4
Phe	101.4	± 21.8

Table 4: Amino acid concentration of valine, homocysteine, methionine, allo-isoleucine, isoleucine, leucine, tyrosine, and phenylalanine in nmol/mL plasma of patients with Diabetes Type 2.

Amino Acid	Mean $\bar{x}$	SD
Val	218.6	± 24.3
H-Cysteine	19.6	± 2.6
Met	19.1	± 2.1
Ile	73.4	± 13.2
Leu	143.5	± 20.6
Tyr	74.9	± 15.1
Phe	56.9	± 7.2

References

- Metabolite Profiles and the Risk of Developing Diabetes. Thomas J. Wang, et. al. Nat Med. 2011 April; 17(4): 448–453.
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