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Metabolomics-based treatment for chronic diseases: results from a multidisciplinary clinical study

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ABSTRACT

Background Non-communicable diseases (NCDs), known as chronic diseases, significantly impact patients' quality of life (QoL) and increase medical expenses. The majority of risk factors are modifiable, and metabolomics has been suggested as a promising strategy for their evaluation, though real-world data are scarce. This study evaluated the QoL improvement and cost-effectiveness of a metabolomics-based treatment for NCDs, aiming to restore metabolic dysfunctions and nutritional deficiencies.

Methods We performed a pre–post intervention analysis using clinical, metabolomics, QoL and economic data obtained from the electronic health records of 765 patients visiting a private practice. The intervention consisted of personalised treatment to restore metabolic dysfunctions and nutritional deficiencies identified by metabolomics alongside the standard treatment for their condition. The mean intervention duration was 401 days.

Results Significant improvement was identified in energy levels, sleep quality, gastrointestinal function and physical activity ($p<0.001$). 67.9% of participants reported significant improvement in the overall QoL, and the average quality-adjusted life-years (QALYs) increased by 0.064 (95% uncertainty interval 0.050 to 0.078) post-treatment. The incremental cost-effectiveness ratio was estimated at €49.774/QALY (95% CI €40.110 to €61.433). Metabolic profiling demonstrated that 16/35 organic acids and 11/24 total fatty acids were significantly changed post-treatment ($p<0.001$), participating in key pathways such as energy metabolism, microbiome and neurotransmitter turnover. Vitamin D and 5-methyltetrahydrofolate insufficiency was significantly restored ($p=0.036$).

Conclusion This is the first study providing evidence that the integration of metabolomics in clinical practice can have a clinical benefit for patients' QoL and may be a cost-effective method.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The rise of non-communicable diseases (NCDs), a major health challenge and economic burden, is mainly driven by modifiable risk factors (unhealthy diet, physical inactivity, etc).
⇒ Patients with NCDs experience a reduced quality of life (QoL) due to metabolic complications or co-morbidities that cannot be addressed by standard treatments.
⇒ Metabolomics detects metabolic changes resulting from genetic and modifiable risk factors interaction, providing insight into personalised treatment.

WHAT THIS STUDY ADDS

⇒ This study found that patients receiving metabolomics-based treatment had improved QoL and that the intervention might be cost-effective.
⇒ The pre–post treatment analysis is based on real-world data and metabolomics, linking the metabolic changes with patients' QoL, which is an understudied field.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This is the first pre–post study on the effectiveness and cost-effectiveness of metabolomics application in NCDs setting the grounds for further research.
⇒ Metabolomics-based treatments should be further assessed as part of personalised treatment strategies against NCDs.

INTRODUCTION

Non-communicable diseases (NCDs), including cardiometabolic conditions, autoimmune diseases and cancer, are chronic diseases primarily influenced by non-genetic modifiable factors. Thus, their control could prevent 80% of NCD-related deaths.[1 2] Among the most important contributing factors are unhealthy diet and micronutrient deficiency, lack of exercise, alcohol abuse and socioeconomic factors.[3] Poor dietary patterns are responsible for more deaths than tobacco or any other health risk. However, they are not assessed by physicians adequately.[4] In addition, current treatment approaches have only proven beneficial for some patients (30%–60%) and do not consider individual risk factors.[5] As a result, patients with NCDs often do not meet their health goals, either due to the low responsiveness and drug adverse effects or related complications. Specifically, increased rates of comorbidity

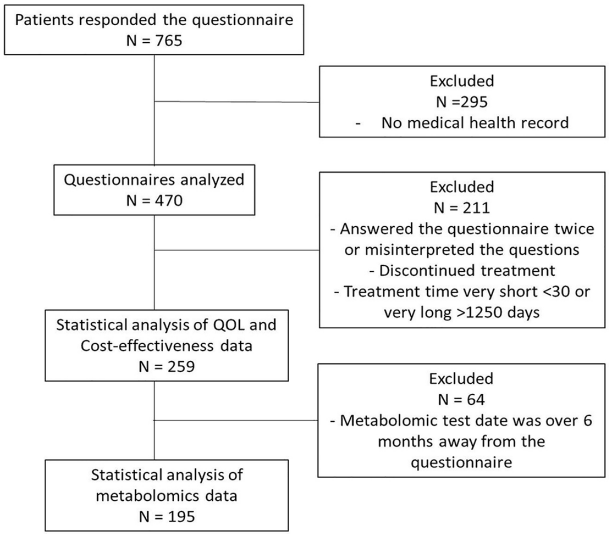

Figure 1 Study design diagram. QOL, quality of life.

and the presence of associated metabolic complications deteriorate the overall quality of life (QoL) of patients. For instance, patients with Hashimoto's thyroiditis, the most common autoimmune disease, suffer from fatigue, weight gain, gastrointestinal problems, depression, cognitive impairment, and muscle and joint pain.[6 7] These symptoms are reported even in some treated patients with normal thyroid-stimulating hormone levels (TSH), suggesting that other factors should be evaluated.[8 9] These QoL aspects are similarly affected in other NCDs such as inflammatory bowel disease and rheumatoid arthritis.[10 11]

Besides the health challenge, NCDs are a significant economic burden to healthcare systems, patients and society. For instance, in the USA, the direct cost of chronic diseases has been estimated at US$216 billion annually, causing US$147 billion in lost productivity, while in Europe, premature death from chronic diseases in the working-age population is responsible for €115 billion loss yearly.[12]

Overall, there is a pressing demand for effective and patient-centred solutions to improve patient's QoL by leveraging recent medical and technological advances.

Precision medicine considers an individual's gene variability, lifestyle and nutrition for early diagnosis and personalised treatment incorporating—omics technologies.[13 14] Metabolomics is the study of metabolites within an organism and provides insights into cellular metabolic health and micronutrient status, giving in-depth information on the phenotype.[15 16] Patients with NCDs are characterised by low-grade inflammation and metabolic complications (such as insulin resistance), and their evaluation represents one of the clinical applications of metabolomics.[17 18]

Here, we report the findings of a pre–post study of patients with chronic diseases who received metabolomics-based treatment focusing on metabolic changes and the associated improvement in the patient's QoL. An economic analysis was also undertaken to investigate the cost-effectiveness of the metabolomics-based approach.

Table 1 Patient's characteristics used in the analysis

	Hashimoto	Other indications
Gender n (%)		
All	84 (100)	175 (100)
Male	9 (10.7)	64 (36.6)
Female	74 (89.3)	111 (63.4)
Age years (SD)		
All	50 (10.5)	47.4 (13.4)
Male	47.2 (18.5)	47.3 (14.8)
Female	50.3 (9.3)	47.4 (12.6)
BMI (SD)		
All	26.5 (5.8)	25.4 (4.8)
Male	26.8 (6.0)	26.1 (4.0)
Female	26.4 (5.8)	25.0 (5.2)
Smoking n (%)		
All	20 (23.8)	37 (21.1)
Male	–	15 (40.5)
Female	20 (26.6)	22 (59.5)
Alcohol consumption		
All	4 (4.7)	22 (12.6)
Male	1 (11.1)	12 (18.8)
Female	3 (4.0)	10 (9)
Days of intervention (SD)		
All	382 (308)	410 (317)
Male	300 (169)	424 (314)
Female	392 (320)	402 (319)

*Alcohol consumption refers to >3 drinks per week.
BMI, body mass index.

MATERIALS AND METHODS

Study design

The present study employed retrospective pre–post data from the electronic health records platform of the 'health clinic for autoimmune and chronic diseases' in Athens, Greece. Initial screening of 765 individuals who visited the practice, from 15 June 2016 to 15 March 2023, and had responded to the QoL questionnaires after receiving treatment recommendations (QoL at T1), was conducted. Data analysis was performed on participants for whom thorough medical records, follow-up responses and metabolomic data were available (figure 1). All the available data of the subjects were collected and assessed by physicians and biologists. Baseline QoL levels (QoL at T0) were calculated based on the patient's medical history, which was matched to questionnaire scaling by experienced physicians according to the scale shown in online supplemental table 1. Metabolite levels were also compared between the two time points.

Study population

Eligible criteria for the study's participants were male and female adults 18–65 years with a diagnosed chronic disease or related symptomatology who had performed metabolomic analysis and received treatment. Exclusion

Table 2 Quality of life changes after metabolomics-based treatment

	B-Mean	B-SD	B-Min	B-Max	95% LCI	95% UCI
Energy level (T0)	57.7%	1.3%	52.6%	62.3%	55.3%	60.1%
Energy level (T1)	72.1%	1.1%	68.5%	76.1%	70.1%	74.3%
Difference gain*	14.4%	1.3%	9.6%	19.1%	**11.8%**	**17.2%**
Sleep quality and duration (T0)	64.1%	1.2%	59.9%	68.4%	62.0%	66.4%
Sleep quality and duration (T1)	72.0%	1.2%	67.7%	75.7%	69.7%	74.1%
Difference gain*	7.9%	1.3%	2.7%	12.1%	**5.3%**	**10.5%**
Gastrointestinal function (T0)	61.6%	1.3%	55.4%	66.0%	59.4%	64.3%
Gastrointestinal function (T1)	74.3%	1.2%	69.0%	78.5%	71.7%	76.5%
Difference gain*	12.6%	1.4%	7.0%	17.3%	**10.0%**	**15.2%**
PAL (T0)	54.1%	1.5%	48.2%	59.8%	51.4%	56.8%
PAL (T1)	66.9%	1.2%	63.0%	71.0%	64.6%	69.3%
Difference gain*	12.7%	1.6%	5.6%	19.0%	**9.6%**	**16.2%**
Utility Score_(T0)	59.4%	0.8%	56.3%	62.1%	57.9%	61.0%
Utility Score_(T1)	71.3%	0.8%	68.3%	74.0%	69.7%	72.8%
Difference gain*	**11.9%**	**0.9%**	**8.9%**	**15.3%**	**10.2%**	**13.7%**
QALYs_(T0)	0.671	0.036	0.537	0.798	0.603	0.744
QALYs_(T1)	0.735	0.037	0.592	0.869	0.666	0.809
QALYs_gained*	**0.064**	**0.007**	**0.035**	**0.091**	**0.050**	**0.078**

Results were based on 5000 bootstrap experiments.
*Difference was statistically significant in the 95% level of significance shown in bold (p<0.001).
B, bootstrap; LCI, lower CI; PAL, physical activity level; QALYs, quality-adjusted life-years; UCI, upper CI.

criteria for the participants were athletes, obese, pregnant or lactating women and individuals diagnosed with cancer or have an infectious disease. Individuals who answered the questionnaire twice, misinterpreted the questions, did not adhere to treatment or were treated for less than 30 days were also excluded. Newly diagnosed patients were not included.

Metabolomics-based treatment

The process for the metabolomics-based treatment includes:

1. Collection of medical and nutritional history from the physician and nutritionist, respectively.
2. Biochemical testing and common blood tests.
3. Metabolomic analysis of organic acids, fatty acids and serum metabolites.
4. Treatment customisation (standard and metabolomics-based intervention).
5. Interim assessment of adherence and efficiency to optimise treatment.
6. Follow-up.

Customisation of metabolomics-based treatment was based on reference and optimum values of metabolically healthy individuals as assessed by the clinic's database of 40 000 metabolomic tests and published databases of reference values.[19–22] Treatment consisted of the standard treatment (surgery, medication and other treatments) and a metabolomics-based intervention to tackle metabolic dysfunctions and nutritional deficiencies (for details, please see online supplemental methods and table 2).

Targeted metabolomics

Metabolomics was applied for the quantification of organic acids, fatty acids and serum metabolites based on previously published work[19 23] (for details see online supplemental material). The interval between metabolomic analysis was defined as the number of days between the first metabolomic analysis of the participants and the metabolomic analysis near the day the participants answered the questionnaire (T_1). The selected metabolomic analysis, conducted within a 6 month window before or after the day the questionnaire was completed, included the largest amount of metabolomic data. The metabolomic dataset was screened for outliers in each variable by experienced staff, and none was deleted as the sample was considered representative.

Questionnaire and modelling of the utility values

A generic, self-reported, four-dimensioned, custom questionnaire was developed internally by the clinical experts of our team leveraging the clinical experience and previously published work as described in online supplemental material. Briefly, four main categories were assessed, namely (a) the level of the patient's energy, (b) the sleep quality, (c) the gastrointestinal function and (d) the physical activity levels (PAL).

Utility values refer to the quality of a patient's life (QoL) associated with different health states. A quality-adjusted life-years (QALYs) is a measure combining life expectancy and QoL and represents the standard approach in economic evaluations.[24] QALYs calculation is the treatment time multiplied by the total utility score, which, in the present analysis, was represented by the total score of the questionnaire. This assumption was based on the disease-specific utility values obtained by the related literature and the baseline utility values from our dataset that were considered reasonably similar by the physicians.[25] It was conservatively assumed that patients who would stop treatment would have the convergence of utility back to baseline in 1 month. The study also investigated aspects of utility including knee pain, decrease in headache frequency, stress levels, decrease in exacerbation frequency.

Perspective of economic analysis

The costs and benefits were recorded and assessed to form the perspective of analysis. In the present study, since metabolomic-based treatments are not reimbursed by the state health system, a patient perspective was adopted, including all medical costs and focusing on their payments. Other types of indirect costs, such as productivity loss, were not considered.

Economic analysis and costing methodology

The incremental cost-effectiveness ratio (ICER), the cost-effectiveness acceptability curve (CEAC) and the expected-value-of-perfect-information (EVPI) curve were estimated. CEAC determines the probability that the combination of standard treatment and metabolomics-based intervention may represent a cost-effective approach for a range of maximum willing-to-pay thresholds per QALY.[26] The EVPI estimates the value of simultaneously eliminating all the uncertainty and thus provides the decision-maker with an indication of the value of collecting additional information.[27] Total costs included (a) the cost of metabolomic exams and (b) the cost of supplements (online supplemental table 3). The cost of main drugs and the cost of adverse reactions were not taken into consideration.[28 29] A modelling approach was used for the final calculation of total cost. In particular, an 80% compliance of patients was assumed based on data from a large systematic review of oral supplements.[30] To deal with uncertainty in probabilistic analysis, the bootstrap method was conducted to estimate standard errors and the 95% CIs with the use of a straightforward percentile method. All price data referred to the economic year 2023. Prices used in the model for nutritional supplements were extracted by the private sector and might be considered common across the country. The impact of patient characteristics on total cost was investigated within a generalised linear regression framework.[31]

Statistical analysis

Analyses were conducted by using VBA Excel, IBM SPSS V.22 software[32] and the web-based MetaboAnalyst V.5.0 platform.[33] Data processing included noise reduction, outlier detection, the rescaling of compounds and the normalisation and retransformation of the data. Outlier detection was conducted graphically and assessed for typo errors by our team's clinicians. Data filtering was based on the inter quartile ratio. All data were log-transformed, autoscaled and centred with the median, while compounds that had more than 50% missing values were excluded from the analysis. We also replaced the rest of missing values using one-fifth of the minimum positive value of each compound. Normality was investigated via the quantile-quantile plots. Univariate statistical analysis was used to detect differences between organic acids (OA), fatty acids(FA) between pre- and post- metabolomic-based treatment.[34] P values were determined for Wilcoxon rank-sum test, as a paired sample, assuming unequal group variance with false rate detection. Due to multiple comparisons, the false discovery rate (FDR) method was used to limit the falsely detected 'significant' results to 5%.[35] Fold change (FC) analysis was also conducted to measure changes between the baseline and the subsequent measurement after intervention, and volcano plots were used to investigate statistical significance (p value) versus magnitude of the FC threshold >1.5).[36] Variables were considered statistically significant if they satisfied the criteria for both metrics, as determined by the relevant statistical tests.

RESULTS

Quality of life

259 patients were included in the study and their subject characteristics and demographics are reported in table 1. The main results of the model concerning the effect size of the improvement compared with the baseline QoL are presented in table 2. In short, the intervention had a statistically significant improvement (p<0.001) to all categories tested (energy, gastrointestinal function, sleep quality and physical activity) with an 11.2% (95% CI: 10.2% to 13.7%) combined improvement on average. More remarkable improvement was observed in the energy level of patients, while the lowest improvement was observed in sleep quality. Results of the supplementary analysis indicated that the level of knee pain was improved by 3.4% (95% CI: 0.2% to 5.9% p<0.001) before and after treatment while was also improved by 7.2% (95% CI: 5.4% to 9.0%, p<0.001) and 6.4% (95% CI: 5.4% to 9.0%, p<0.001) concerning the frequency of exacerbations and the stress level, respectively. Differences at the headache level −2.1% (95% CI: −4.9% to 0.8%, p=0.160) before and after treatment did not reach statistical significance. Questionnaire data analysis was performed to assess the extent of the effect (% of patients). Energy levels were improved in 58.3% of patients, 46.7% had at least a 20% improvement in sleep quality and duration, 60% showed

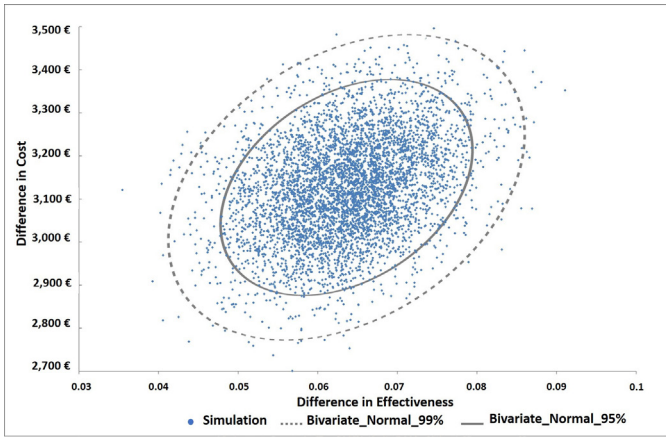

Figure 2 Scatter plot of metabolomics-based treatment versus conventional approach based on probabilistic analysis. The figure shows the results of 5000 simulations, where each dot represents a different probabilistic experiment of bootstrap method. The depicted ellipse's contour followed the bivariate normal distribution estimating the 95% and 99% uncertainty intervals.

improved gastrointestinal function and 54.4% showed improved PAL. A maximum of 18% of patients reported a decline in any of these areas, while 28.2%–36.3% experienced a consistent QoL throughout treatment (online supplemental table 4). When requested to assess their overall improvement, 95.7% of patients reported some improvement in their QoL after treatment, with 67.9% experiencing significant improvement.

Cost-effectiveness analysis

The economic data for the determination of the cost of intervention are listed in online supplemental table 3. Overall, the cost of the intervention per patient was estimated at €3127 (95% CI: €2901 to €3367) for a mean duration of treatment at 401 days (95% CI: 365 to 439) or a cost of €7.8 per day per patient (95% CI: €7.3 to €8.2) (online supplemental table 5). The cost of supplements accounted for 52.1%, while the cost of metabolomic exams accounted for 47.9%. The ICER was estimated

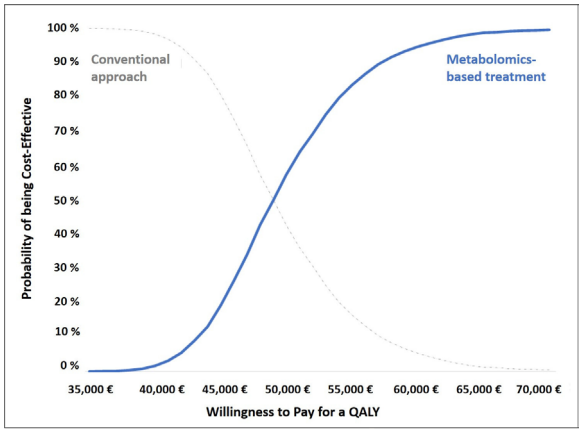

Figure 3 Cost-effectiveness acceptability curve of metabolomics-based versus conventional approach. QALY, quality-adjusted life-years.

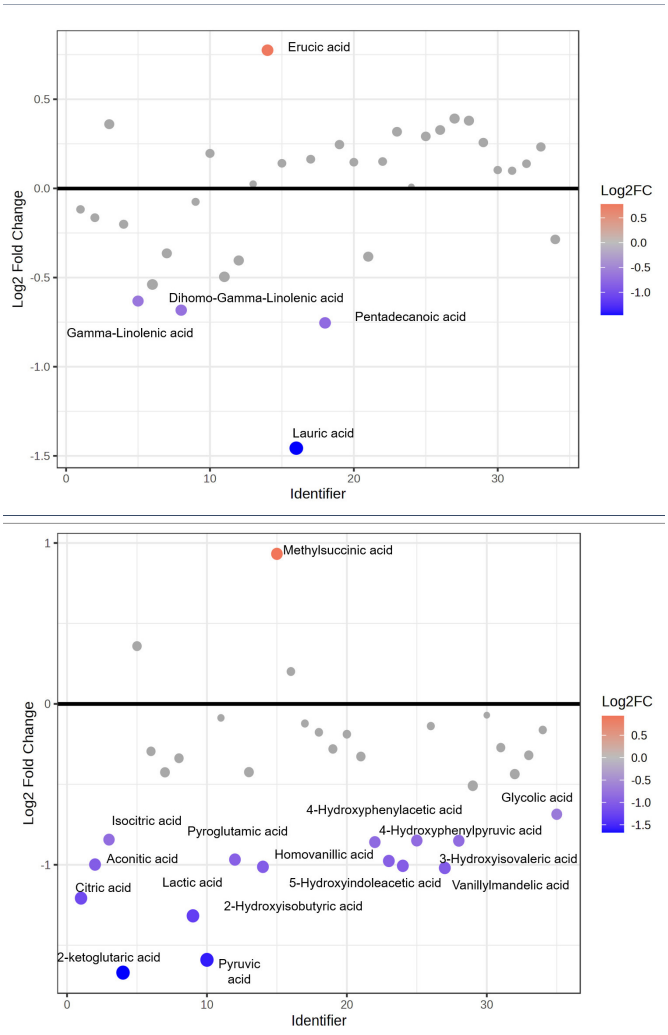

Figure 4 Fold change (FC) analysis of the fatty acid (up) and the organic acid (down) metabolic compounds before and after metabolomic treatment (FDR<0.05, FC>1.5). FDR, false discovery rate.

at €49.774/QALY (95% CI €40 110 to €61 433). The estimated mean number of QALYs after the intervention was 0.735 (95% uncertainty interval (UI) 0.666 to 0.809) compared with 0.671 (95% UI 0.603 to 0.774) at baseline. Consequently, there was a statistically significant difference of 0.064 (95% UI 0.050 to 0.078) QALYs after treatment. Online supplemental table 6 shows the results of the generalised linear model that depicts the association between the cost of the intervention and the patient's characteristics. Statistically significant variables were the duration of treatment and the baseline utility in the 90% significance level. The remaining variables (ie, age, body mass index, smoking status, gender and disease) were excluded as non-statistically significant. Analyses revealed that the metabolomics-based treatment was more effective than its comparator, with a probability of more than 99.9%, but also more costly (figure 2). Willingness to pay for a QALY within the range of €50 000–€60 000 is the most favourable to characterise the intervention as 'cost-effective'). In particular, willingness to pay at €60 000

determines a probability for being cost-effective at 95% (figure 3). The cost of information (EVPI analysis) for €40 000/QALY, €50 000/QALY and €60 000/QALY was determined at €584, €113 and €10, respectively (online supplemental figure 2).

Metabolomic profiling

In total, 32 total fatty acids and 37 organic acids were measured in a group of 195 patients. Values of mean±SD and median for each variable are presented in online supplemental tables 7,8. In total, 16 metabolites were found to be statistically significant before and after treatment for OA, and 18 were significant for FAs, under the assumption of non-difference. To illustrate the clinical interpretation of these findings, the metabolic pathways of OA and FA are included. Fold change analysis determined that 16 OA and 5 FA were statistically significantly altered post-treatment (FDR<0.05, fold change (FC)>1.5) depicted in figure 4. Similar results were obtained from volcano plot analysis (online supplemental figure 3). Collectively, quantitative analysis of OA showed a significant change in the levels of 16 metabolites even when adjusted for multiple correlations using the FDR. To gain meaningful insight into these findings, a quantitative enrichment analysis was conducted using MetaboAnalyst V.5.0. In online supplemental figure 4, the metabolic pathways with significant changes (p<0.05) and represented by a large number of metabolites (enrichment) are shown, based on the OA concentration levels. Levels of vitamin D were statistically significantly higher compared with baseline (p=0.0036 (mean±SD) 28.4±11 vs 58.6±22.5 ng/mL), leading to vitamin D correction from 48.1% (<30 ng/mL) to 1.2% patients with low vitamin D levels post-treatment. Similarly, 5-methyltetrahydrofolate levels were increased from 14.15±4.8 to 26.6±8.3 nmoL/L (p=0036) in patients who received the treatment.

DISCUSSION

NCDs have a dramatic impact on patients and society, especially in cases of multimorbidity, which accounts for 35% of cases and 51% in older individuals.[37] Despite the diverse clinical symptomatology, NCDs share common pathogenetic mechanisms and risk factors, which can be evaluated through metabolomics, with the potential to improve the long-term disease course and QoL.[17]

In the present work, we assessed the effectiveness and cost-effectiveness of a metabolomic-based intervention to target common metabolic dysfunctions and improve the QoL of patients with chronic diseases. To the best of our knowledge, this is the first study to assess the applicability and clinical utility of metabolomics on chronic diseases.

Metabolomics-based treatment improved levels of energy and physical activity, sleep quality and gastrointestinal function, which had been considered as the most important by the patients. Fatigue is prevalent in almost all autoimmune and chronic diseases, including autoimmune thyroiditis, rheumatoid arthritis and IBD associated with their complex aetiopathogeneis.[7 11] The multifactorial nature of fatigue includes metabolic abnormalities and nutritional deficiencies causing mitochondrial dysfunction, alteration in the neurotransmitter balance and interaction with the microbiome in a proinflammatory state.

Previous supplementation studies have demonstrated a beneficial effect of individual supplements on fatigue levels assessed by Fatigue questionnaires.[38] Here, we show that 58.3% of patients had significantly less fatigue compared with baseline levels, and we discuss the potential role of metabolic biomarkers. Specifically, levels of citric acid were markedly increased, followed by cis-aconitic acid, isocitric acid and 2-ketoglutaric acid (p<0.001). As depicted in online supplemental figure 1, these metabolites participate in the first steps of the TCA cycle, supplied by acetyl-CoA which is the key molecule that facilitates glycolytic pyruvate to enter the cycle. These metabolic reactions are sensitive to the abundance of several cofactors, which were included in the metabolomics-based treatment. Therefore, the reduction in lactate and pyruvate levels, along with the increase in the levels of the first TCA metabolites post-treatment, indicates an improved TCA cycle supply. This enhancement of mitochondrial function likely leads to increased ATP production, which may contribute to the observed reduction in patient fatigue.[39] The remaining Krebs cycle metabolites were found to decrease post-treatment, suggesting an unresolved blockade of these pathways requiring additional intervention.[40] These findings are in line with previous studies showing significant TCA cycle disturbance in patients with fatigue syndrome and that exogenous supplementation leads to their increase.[38 41] In addition, we have demonstrated a substantial increase in biomarkers of neurotransmitter metabolism, including the metabolites of dopamine (homovanillic acid), serotonin (5-hydroxyindoloacetic acid) and epinephrine (vanillylmandelic acid). Low levels of these metabolites have been associated with fatigue and depression, though it is important to note that for the dopamine/serotonin ratio, a range of over one should be maintained since high serotonin levels are related to low performance and energy.[42] Neurotransmitter metabolism changes may also be associated with the improved quality of sleep in our population, which was assessed by the custom questionnaire and is in line with previous supporting data.[43] Regarding the improved responses for gastrointestinal function, we observed that microbiome metabolic markers 4-hydroxyphenylacetic acid and 3-hydroxyisovaleric acid were significantly decreased post-treatment (p<0.001). 4-hydroxyphenylacetic acid is an L-tyrosine metabolite synthesised by intestinal microbiota, reflecting the overgrowth of anaerobic bacteria when present at high levels.[44] Similarly, 3-hydroxyisovaleric acid is an early marker of biotin-producing bacteria, and increased levels are associated with biotin deficiency and dysbiosis. Pyroglutamic acid and 2-hydroxybutyric acid (2-HB) are markers of glutathione synthesis and turnover, while 4-hydroxyphenylpuryvic levels are associated

with antioxidant mechanism capacity. All three metabolites were significantly reduced (even though 2-HB was not statistically significantly changed), indicating that metabolomics-based treatment contributed to the antioxidant defence and reduced oxidative stress. Pre–post treatment comparisons of fatty acids revealed a significant increase in polyunsaturated omega-3 fatty acids known for their anti-inflammatory properties discussed previously.[45] In addition, dihomo-gamma-linolenic acid, the precursor of proinflammatory mediators and metabolic biomarkers of insulin resistance, was reduced ($p<0.001$). The ratio of arachidonic acid/eicosapentaenoic acid was significantly lowered post-treatment indicating an improved inflammatory profile of these patients.

Over the years, several studies have demonstrated a beneficial effect of neutraceuticals against NCDs though results remain inconclusive, hampering their translation to clinical practice. As discussed elsewhere, the design of the administered intervention (single or combinatorial) and the selection of the study population (baseline metabolic traits) are critical for interpreting the results.[46] This is due to the multifactorial nature of NCDs and high variability in micronutrient needs among patients, requiring a detailed monitoring tool to track baseline requirements and treatment response. Although it is important not to extrapolate our findings, the present study paves the way for future research into the application of metabolomics in customising treatment against NCDs and tracking metabolic and phenotypic changes.

Overall, applying the clinical and metabolomic data of the present pre–post study in our model resulted in a savings of 0.064 QALYs or 23 days of perfect health with an acceptable ICER.[47–49] The estimation of effectiveness and costs here was restricted within the above-mentioned time horizon, avoiding any statistical extrapolation beyond the end of the study. This approach was considered the most suitable to avoid heavy statistical modelling and thus the adoption of restrictive and sensitive assumptions such as those frequently used in long-term models. Hence, our estimations represent a rather conservative and more realistic approach and might become even more favourable in future metabolomics studies.

One of the strengths of this study is the integration of real-world data from various sources to obtain a better overview of the benefit of metabolomics for the patient. A significant challenge for metabolomic results translation into clinical practice is the lack of a unified methodology across laboratories. To overcome this, we have employed an in-depth quality assurance protocol to assess metabolomics data on a patient level and across the Greek population (detailed in the 'Methods' section). The present analysis has certain limitations. At first, this pre–post study does not consider other contributors, and the results of the intervention cannot be entirely attributed to it. In addition, since the results represent a combinational effect of technologies used, the analysis may incorporate a bias factor. In addition, the population of the study consisted of participants with various diseases which could affect the analysis and should be explored in larger studies. However, considering their common immunometabolic and molecular pathways, addressing their metabolic hallmarks emerges as a potential strategy.[50] Utility weights may not fully represent the entire population. A considerable variation in reported utilities associated with measurement instruments still exists, while similar individuals frequently report different levels of QoL. This is a common issue and partly affects our questionnaire and the present study. Finally, the analysis was conducted from a sickness fund perspective and not the society overall and thus a broader (societal) analysis may be worthwhile since—frequently—indirect patients' costs are higher than the direct ones.

In conclusion, NCDs have a significant impact on a patient's QoL. However, this aspect of NCDs is often overlooked, with more emphasis placed on diagnosis, treatment and managing symptoms. With the advent of advanced technologies, the focus has shifted to personalising treatments to achieve better outcomes and manage diseases more effectively, including related comorbidities. Metabolomics can identify dysfunctional molecular networks leading to a phenotype, whether it is caused by an NCD or multimorbidity. However, the integration of metabolomics in clinical practice is being hampered by the need for a multidisciplinary team consisting of medical doctors, biologists and nutritionists to develop the treatment plan. Further research should focus on the translation and integration of existing knowledge around chronic disease physiology and state-of-the-art methods to their management as well as the development of effective strategies for their wide application.

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Contributors DT and ES conceived and designed the study. VF conducted the analysis, prepared the figures and tables and wrote the manuscript. ES interpreted the results, wrote the manuscript and prepared the figures. SX and DT wrote the manuscript and provided valuable comments in the discussion section. EParamera and EPapakonstantinou supervised GC-MS experiments. SX, MC and MM involved in collecting and managing the personal data of the participants and interpreted the results. AT involved in taking ethical clearance, critically assessed the design of the study and the manuscript. DT is the guarantor. All authors reviewed the manuscript.

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Competing interests DT is a scientific advisor for Meetab Srl and Natural Doctor SA and a scientific director at Metabolomic Medicine. ES, SX, MC and MM work at Metabolomic Medicine SA, VF is an external collaborator of Metabolomic Medicine SA. There is no financial relationship or conflicting interests between the remaining study investigators and the companies Natural Doctor and Meetab Srl. The companies Meetab Srl and Natural Doctor did not interfere in the design, conduct and analysis of the research. The rest of the authors declare no conflict of interest.

Patient consent for publication Consent obtained directly from patient(s).

Ethics approval The participants' data in the study were processed and anonymised in accordance with the EU General Data Protection Regulation (GDPR). Prior to the study, all participants provided informed consent. The study was conducted in compliance with the ethical standards set forth in the 1964 Declaration of Helsinki, or comparable ethical standards, and was approved by the scientific board of the 'Health Clinic for Autoimmune and Chronic Diseases' as well as the Ethics Committee of the University of Crete (approval no. A.P. 63_22032019).

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Data availability statement Data are available on reasonable request. The dataset presented in this study is available to be granted anonymised from the corresponding author on reasonable request.

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