HTB Rejuvenate™ Immuno-Rejuvenation Observational Trial

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Abstract

This observational trial examined the subjective impact of HTB Rejuvenate™, a phytochemical-rich dietary supplement, on immune-related health. Eighteen apparently healthy individuals (14 women, 4 men; average age 61±7.5 years) consumed two capsules twice daily (a total of two thousand milligrams) of a dietary supplement formulated with a Himalayan Tartary buckwheat flour blend containing a concentration of its bioactive phytochemicals (HTB Rejuvenate™) for 30 days. Principal bioactive molecules found in the studied supplement included rutin, quercetin, hesperidin, luteolin, diosmin, 2-hydroxybenzylamine (2-HOBA), and hydroxymethylbutyrate (HMB). In previous studies, these molecules have been found to play a role in adaptive immunomodulation, including support for apoptosis, autophagy, and mitophagy processes. Participants simultaneously tracked their perceived health status using a variety of health questionnaires, which included the Medical Outcome 36 Survey (MOS SF36), the NIH Patient-Reported Outcome Measurement Information System (PROMIS), the Medical Symptoms Questionnaire (MSQ), and a novel questionnaire designed to correlate phenotypes of immune dysfunction with common health conditions (the Immuno-Identity™ Questionnaire). Compared to the start of the study, paired t-test analysis revealed a significant improvement in scores on the Immuno-Identity™ Questionnaire and the Medical Symptoms Questionnaire at the end of the 30-day study period. There was a strong statistical correlation between the improvement in scores after the 30-day intervention on the Immuno-Identity™ Questionnaire and the Medical Symptom Questionnaire. The most common functional improvements were seen in energy, vitality, sleep, mood, pain, and cognition.

Background

It is well recognized that the immune system controls function far beyond the protection against infectious disease.[1] Scientific research increasingly supports the involvement of the immune system on diverse aspects of human health and suggests a role for targeted immune modulation in a variety of pathologies, including cancer, cardiovascular disease, mood disorders, autoimmune disease, and infectious disease.[2] The influence of the immune system on overall health is in part a result of its presence in virtually every organ and tissue of the body, including the blood, intestinal tract, liver, adipose tissue, brain, muscle, respiratory tract, and connective tissue. A better understanding of underlying immunological patterns and their correlations with existing pathological health patterns may help to guide immunological interventions. In addition to pharmaceutical therapies, dietary and supplement-based interventions including vitamins, minerals, and phytochemicals may help shift immunological status towards a more adaptive state.

The makeup and function of the immune system has been shown to reflect and integrate a wide variety of environmental and dietary influences. Alterations in immune system function has been associated with a wide array of symptoms and health-related issues. It is also known that immune system function varies widely among individuals, and this variation is largely driven by non-heritable influences.[3,4] This suggests that an individual's diet, environment, and other lifestyle factors may have a dominant role in "training" the immune system and could perhaps be more significant than genetic inheritance.

Factors Associated with Immunological Resilience

Aging is associated with decreased immune resilience to infectious and inflammatory diseases. It is now recognized that it is not just the conventional concept of chronological age (as measured in birthdays), but rather the biological age of the immune system that influences its function.[5] Whereas chronological age is not modifiable, it has been shown that the

biological age of the immune system can be significantly influenced by our choices.[6] The process of reducing the biological age of the immune system for improved health has been called immune rejuvenation. Applications of immune rejuvenation may help offset age-associated decline in immunological function and associated health issues. Mechanistically, these interventions aim to reduce the detrimental effects of age-related immune dysfunction (called immunosenescence), which can develop as a result of mutational injury and abnormal epigenetic imprinting.[7,8]

It has been proposed that acquired immunological variability between individuals as a response to environmental and lifestyle influences can significantly impact immune resilience throughout adulthood. These modifiable influences include exposure to pollutants, chronic stress, sleep disturbances, exercise, and quality of nutrition. This suggests that the function of the immune system is more dependent upon our lifestyle, diet, and environmental exposures than was previously recognized.[9] Patterns of immune dysfunction linked to these modifiable risk factors are also associated with complex symptom profiles, including pain, changes in cognitive function, energy, mood, sleep, vitality, and organ-specific alterations.[10] This has significant implications for novel immune challenges as well, as it has recently been shown that after infection with SARS-CoV-2, individual immune patterns (immunotypes) were predictive of both the types of symptoms experienced and their severity.[11] Increased risk for complications of SARS-CoV-2 infection has been repeatedly documented in those with pre-existing conditions, including hypertension, obesity, diabetes, and vascular dysfunction.[12] In part, this may be mediated by underlying immune alterations associated with these chronic diseases, which could decrease resilience to SARS-CoV-2 infection as the result of an increased burden of acquired immunological dysfunction.

Evidence supports an important role for nutrition in influencing both innate and adaptive immune function.[13,14] The Western diet, which is rich in highly processed foods, has been shown to trigger innate immune system reprogramming, resulting in imbalanced immune function.[15] Rodgers and Collins, from the National Institutes of Health, stated the following in an article in the Journal of the American Medical Association entitled "Precision Nutrition—the Answer to 'What to Eat to Stay Healthy'": "Research to address and mitigate diet-related chronic diseases is especially important in light of the current coronavirus disease 2019 (COVID-19) pandemic, given the increased risks faced by people with these underlying diseases, which disproportionately affect vulnerable populations due to health and social disparities. Exploring how diet and nutritional status modify immune response could help explain some of the variability in COVID-19 morbidity and mortality, even in individuals without diet-related chronic disease."[16]

Modulation of immune system function through diet is thought to result from specific nutrients influencing a number of important biological mechanisms. Immune cell autophagy has recently been identified to be of principal importance in the rejuvenation of immune function and may represent a key pathway by which a healthy diet translates into immune resilience.[17] Autophagy is the process through which cellular components are broken down and recycled. Regulation of autophagy has been found to play a role in a number of immune-related diseases, including infection, certain cancers, cardiovascular diseases, pulmonary diseases, diabetes, and aging.[18] Over the past decade, the role of autophagy as the central process in trafficking events that maintain innate and adaptive immunity has emerged.[19]

Nutritional strategies to support immune autophagy may promote improved resilience against infectious and non-infectious threats. Research has sought to identify specific dietary nutrients responsible for modulating autophagy in the immune system. Of these, flavonoid phytochemicals have been especially highlighted for their effects.[20,21] Evidence suggests that specific flavonoids, such as quercetin, might improve the immune response to viral infections, including coronaviruses.[22,23] The health applications of autophagy-inducing nutrients, which may enhance immune rejuvenation, have subsequently become a focus in the development of food and nutraceutical products.[24]

Himalayan Tartary Buckwheat

Among foods with elevated immune-rejuvenating potential, the ancient buckwheat cultivar Himalayan Tartary buckwheat (Fagopyrum tataricum) has been found to contain a unique repertoire of relevant phytochemicals. Compared to common buckwheat (Fagopyrum esculentum), Himalayan Tartary buckwheat analysis reveals over 100-fold greater levels of the flavonoid rutin and its metabolite quercetin.[25] The notable differential in production of rutin and quercetin between common buckwheat and Himalayan Tartary buckwheat may in part be explained by variance in the genetic structure of these two plants.[26,27]

Quercetin and rutin, which are principal flavonoids found in Himalayan Tartary Buckwheat, have been proposed to improve immune function related to a number of infectious and non-communicable chronic diseases.[28,29] In a recent study, it was reported that Himalayan Tartary buckwheat might influence pathways that are dysregulated in type 2 diabetes, hypertension, and cardiovascular risk factors.[30] A double-blind, cross-over clinical trial evaluating the impact of consuming cookies made with Himalayan Tartary buckwheat flour demonstrated its impact on lowering both cholesterol and inflammation.[31] Another clinical trial with Himalayan Tartary buckwheat in type 2 diabetics with impaired kidney function demonstrated improvement in insulin sensitivity and kidney function.[32,33] In sum, multiple lines of scientific inquiry have demonstrated the potential for the array of phytochemicals

found in Himalayan Tartary buckwheat to have beneficial effects on health, highlighted by the high levels of rutin and quercetin that have influence on immune-specific autophagy.

Based upon this body of literature, a study was designed to evaluate the impact of supplementation with a dietary supplement concentrate of Himalayan Tartary buckwheat flavonoids (HTB Rejuvenate™), delivered in capsule form in a small observational trial. The outcome measures for this 30-day experience trial employed the NIH Patient-Reported Outcomes Measurement Information System (PROMIS), the Medical Outcome Short Form 36 Questionnaire (MOS SF36), the Medical Symptoms Questionnaire (MSQ), and the Immuno-Identity™ Questionnaire.[34,35,36] The Immuno-Identity™ Questionnaire as shown in Figure 1 was developed by the clinical studies group to evaluate specific symptoms associated with the phenotypes associated with various imbalanced immunophenotypes that were classified as angry, confused, sensitive, or withdrawn. Scoring of the questionnaire was done through the use a Likert score hierarchy. The Medical Screening Questionnaire (MSQ) is provided in Figure 2.

Methods

Study Design and patients

Twenty participants were recruited and qualified into the study. Two of the participants dropped out due to time constraints associated with filling out the questionnaires and keeping a daily log. Of the eighteen participants that completed the trial, fourteen were women and four were men. The age range of those who completed the study was 47 to 80 years of age (average 61+/-7 years). All participants were considered to be in good health and free of any specific diseases. They were provided with a detailed description of the study protocol, as well as information about the composition of the nutritional supplement and the safety profile of its ingredients. The health histories of all participants were reviewed by the medical and nutritional members of the study team prior to their inclusion in the study. All participants in the study signed an informed consent form.

Daily dose: Four capsules daily of Himalayan Tartary buckwheat phytochemical concentrate (HTB Rejuvenate™) used in the study provided the following amounts of bioactive phytochemicals:

2000 mg of fortified Himalayan Tartary buckwheat flour containing 150 mg rutin, 400 mg quercetin, 150 mg luteolin, 150 mg hesperidin, 150 mg diosmin, 100 mg 2-hydroxybenzylamine (2-HOBA), 500 mg hydroxymethylbutyrate.

Data Collection

Each participant was provided with the four questionnaires, which they were asked to complete at the beginning of the 30-day trial, at the two-week period, and again at the completion of the trial. The completed questionnaires were returned in a self-mailer to the study data manager at the initiation of the study, and at two-week and four-week intervals, along with daily logs of subjective feelings about health, diet, and activity patterns. This data was entered into a database that allowed for interrogation of the cumulative and individual participate data sets. Compliance with the study was judged on the basis of completion of the questionnaires and use of the nutritional supplement capsules.

Statistical Analysis

Analysis of the results was accomplished by using the paired two-tailed t test for correlation between before and after scores on the questionnaires, both for individual and group differences. An algorithm was developed to evaluate the trend toward or away from a balanced immune system based upon the percent improvement in balanced immune scores versus those immuno-types characterized as having imbalanced immune system function as a result of responses to the Immuno-Identity™ Questionnaire

Results

In evaluation of the four questionnaires used in the trial, it was found that the Medical Symptoms Questionnaire (MSQ) and the Immuno-Identity™ Questionnaire had the greatest sensitivity to changes in immunophenotype of the participants over the 30-day trial. The results of the study indicated that the improvement in the group MSQ scores strongly correlated with improvement in group Immuno-Identity™ Questionnaire scores at a significance of $p<0.0004$. There was also a statistically significant correlation at the $p<0.0051$ between improvement in the starting versus the final immuno-identity scores for the group. The difference between the pre- and post-Immuno-Identity™ Questionnaire scores for each individual were also evaluated, and were found as a group to have improved at a statistically significance of $p<0.0043$.

There were also changes seen in many of the participants in their PROMIS and MOS SF 36 questionnaires, but the differences measured by these instruments did not reach statistical significance. It is possible these questionnaires would be more suited to measuring longer-term changes in immunophenotype, and therefore were not sensitive enough to pick up smaller degrees of short-term improvements in symptoms.

These results of the individual participants in their before and after scores for the Medical Symptoms Questionnaire and the Immuno-Identity™ Questionnaire are shown in Figure 3 and Figure 4.

The information provided by the participants in their daily logs, coupled with the before- and after-intervention with the Himalayan Tartary buckwheat nutritional supplement questionnaire data, indicated that the major

immunophenotypic improvements were related to sleep, energy levels, alertness, and mobility.

Discussion

Given the short duration of the 30-day trial and the small participant number, it was remarkable that the improvements in general immune-related phenotypes were so statistically significant. This suggests the phytochemical composition of the Himalayan Tartary buckwheat supplement exerts a strong biological signal that impacts immune system function. It is recognized that immunophenotypes are correlated with broad symptom signatures, including inflammation, pain, nervous system, skin, digestive, and musculoskeletal issues.[37] Both the Medical Symptoms Questionnaire and the Immuno-Identity™ Questionnaire were capable of assessing this broad range of symptoms using a Likert scale system. While these questionnaires have a number of limitations in determining the precise immunological mechanistic source of an individual's symptoms, they do represent sensitive initial screening tools for broadly defining specific immunophenotypes.[38,39]

With regard to the improvements in energy, mobility, sleep, and alertness that were noted following the 30 days of supplementation, these effects might be related to improvement in immune cell bioenergetics, which is regulated by immune cell mitochondrial function. It has recently been reported that dysfunctional mitochondria in T lymphocyte cells results in a variety of symptoms and premature senescence.[40] Dysfunction of T lymphocyte mitochondria has been reported to produce multiple organ-related symptoms, including metabolic, cognitive, physical, and cardiovascular issues. It has been shown that specific dietary flavonoids play an important role in eliminating damaged mitochondria through the process of mitophagy.[41] The flavonoids that have been demonstrated to enhance mitophagy include those found in high levels in Himalayan Tartary buckwheat.[42,43,44] Recent studies have shown that two important members of the portfolio of flavonoid family in Himalayan Tartary buckwheat—quercetin and rutin have important roles in promoting mitochondrial function in immune cells and serving as "senolytics" in their ability to reduce immune senescence.[45,46,47]

Himalayan Tartary buckwheat also contains the unique phytochemical 2-hydroxybenzylamine (2-HOBA), which has been found to have a beneficial effect on immune system function and a role in regulating blood pressure.[48,49] A recent study indicates that 2-hydroxybenzylamine has a direct effect on mitochondrial function by protecting against oxidative injury.[50] A growing body of research suggests that Himalayan Tartary buckwheat contains a dynamic portfolio of "anti-stress" phytochemicals that protect against immune injury, influence autophagy and mitophagy, have an epigenetic impact on immune cell aging, and stimulate immune rejuvenation.[51,52]

The NU-AGE study was a one-year pilot investigation in 120 elderly healthy subjects in Poland and Italy who followed a Mediterranean diet that was naturally high in flavonoids. The participants in this study had their immune cell age determined by methylation patterns before and after intervention. One finding was evidence of immune rejuvenation, as measured by an alteration in the epigenetic methylation patterns of immune cell genes that reflected a younger biological age at the end of the study. This alteration in methylation patterns was associated with changes in coding regions of the genome that are linked to mitochondrial bioenergetics, regulation of cellular recycling, and immune function.[53] The results of this study support the concept that diets containing high levels of bioactive nutrients are associated with protection against immune cell dysfunction. Although the specific nutrients responsible for this improvement in immune cell age was not ascertained, it is well known that aging of the immune system is related to the epigenetic alteration of methylation patterns of the autophagy genes in macrophage cells.[54] This line of research demonstrates how potentially important a phytochemically-rich diet may be in promoting immune rejuvenation.

The small pilot study presented in this paper—one that was undertaken to examine the influence of a Himalayan Tartary buckwheat phytochemical nutritional supplement (HTB Rejuvenate™) on immune-related symptoms—produced statistically significant outcomes that suggest this product has a very unique influence on the biological processes associated with immune rejuvenation. As an experience trial, this work paves the way for a controlled study on the mechanistic influence of the Himalayan Tartary buckwheat nutritional supplement (HTB Rejuvenate™), including its influence on immune autophagy and mitophagy in people with specific immuno-identities.

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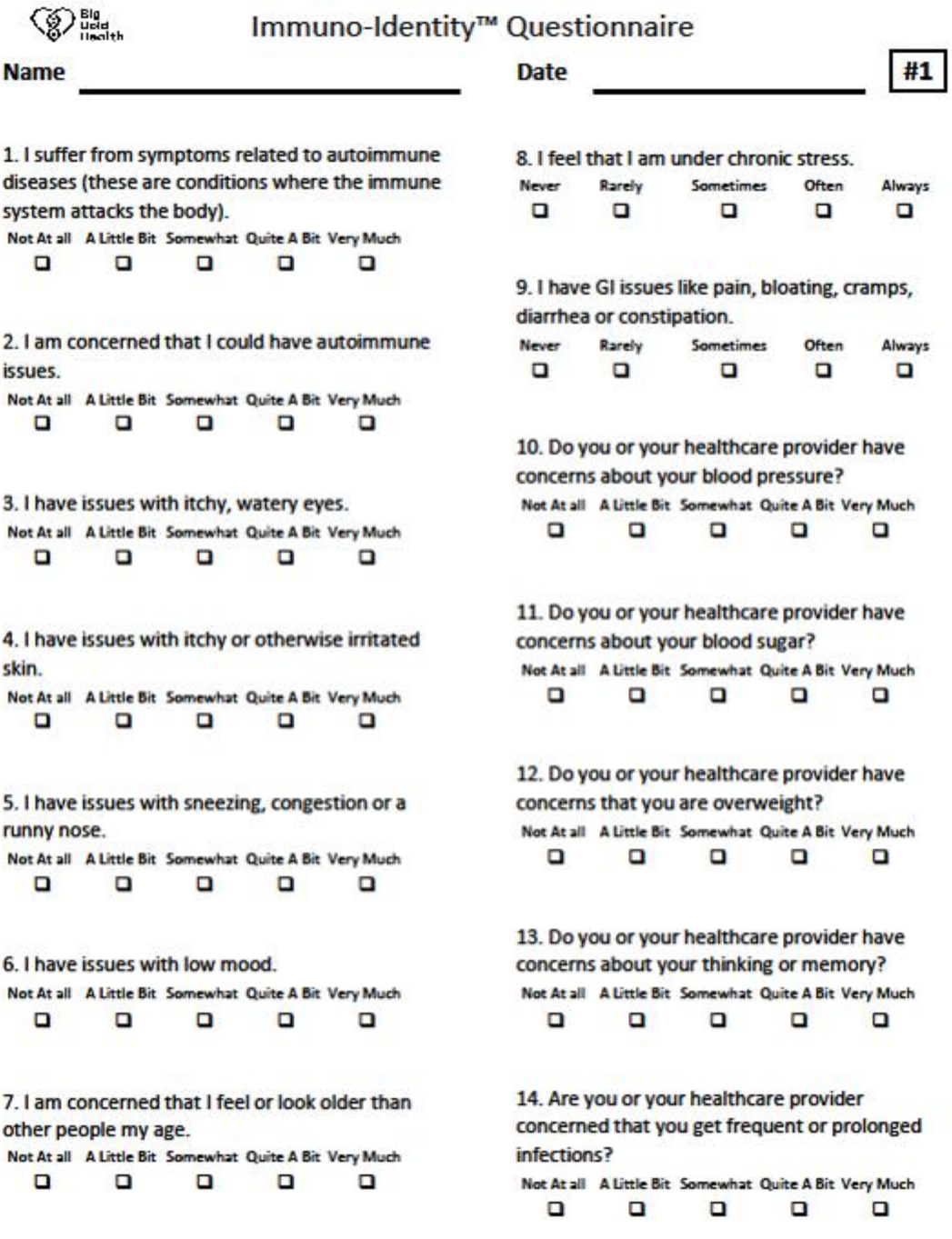
Figure 1. Immuno-Identity™ Questionnaire



Immuno-Identity™ Questionnaire

Name _____ **Date** _____ **#1**

1. I suffer from symptoms related to autoimmune diseases (these are conditions where the immune system attacks the body).

Not At all ☐ A Little Bit ☐ Somewhat ☐ Quite A Bit ☐ Very Much ☐

2. I am concerned that I could have autoimmune issues.

Not At all ☐ A Little Bit ☐ Somewhat ☐ Quite A Bit ☐ Very Much ☐

3. I have issues with itchy, watery eyes.

Not At all ☐ A Little Bit ☐ Somewhat ☐ Quite A Bit ☐ Very Much ☐

4. I have issues with itchy or otherwise irritated skin.

Not At all ☐ A Little Bit ☐ Somewhat ☐ Quite A Bit ☐ Very Much ☐

5. I have issues with sneezing, congestion or a runny nose.

Not At all ☐ A Little Bit ☐ Somewhat ☐ Quite A Bit ☐ Very Much ☐

6. I have issues with low mood.

Not At all ☐ A Little Bit ☐ Somewhat ☐ Quite A Bit ☐ Very Much ☐

7. I am concerned that I feel or look older than other people my age.

Not At all ☐ A Little Bit ☐ Somewhat ☐ Quite A Bit ☐ Very Much ☐

8. I feel that I am under chronic stress.

Never ☐ Rarely ☐ Sometimes ☐ Often ☐ Always ☐

9. I have GI issues like pain, bloating, cramps, diarrhea or constipation.

Never ☐ Rarely ☐ Sometimes ☐ Often ☐ Always ☐

10. Do you or your healthcare provider have concerns about your blood pressure?

Not At all ☐ A Little Bit ☐ Somewhat ☐ Quite A Bit ☐ Very Much ☐

11. Do you or your healthcare provider have concerns about your blood sugar?

Not At all ☐ A Little Bit ☐ Somewhat ☐ Quite A Bit ☐ Very Much ☐

12. Do you or your healthcare provider have concerns that you are overweight?

Not At all ☐ A Little Bit ☐ Somewhat ☐ Quite A Bit ☐ Very Much ☐

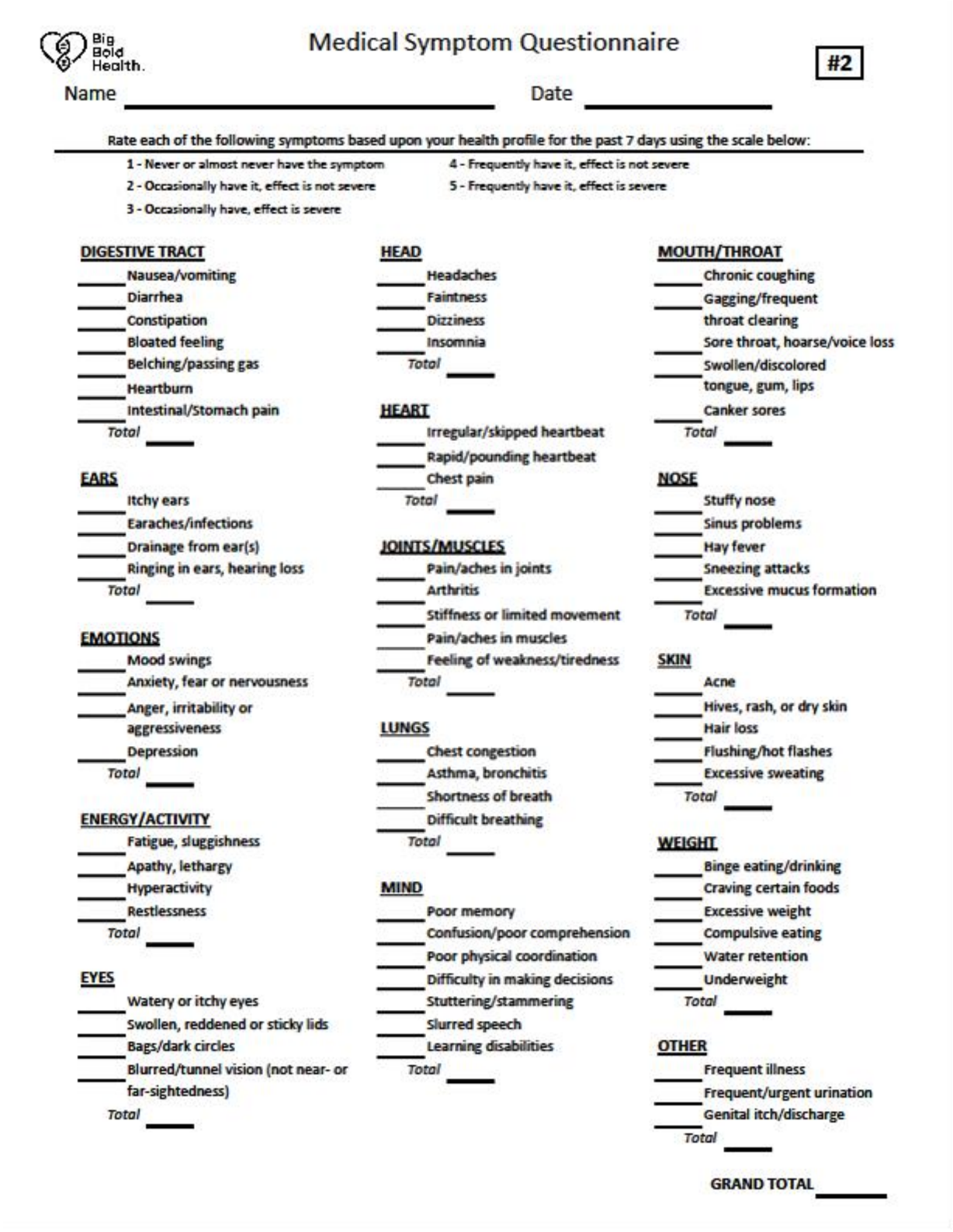
13. Do you or your healthcare provider have concerns about your thinking or memory?

Not At all ☐ A Little Bit ☐ Somewhat ☐ Quite A Bit ☐ Very Much ☐

14. Are you or your healthcare provider concerned that you get frequent or prolonged infections?

Not At all ☐ A Little Bit ☐ Somewhat ☐ Quite A Bit ☐ Very Much ☐

Figure 2. Metabolic Screening Questionnaire



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Medical Symptom Questionnaire

#2

Name _____ Date _____

Rate each of the following symptoms based upon your health profile for the past 7 days using the scale below:

1 - Never or almost never have the symptom
2 - Occasionally have it, effect is not severe
3 - Occasionally have, effect is severe
4 - Frequently have it, effect is not severe
5 - Frequently have it, effect is severe

DIGESTIVE TRACT
_____ Nausea/vomiting
_____ Diarrhea
_____ Constipation
_____ Bloated feeling
_____ Belching/passing gas
_____ Heartburn
_____ Intestinal/Stomach pain
Total _____

EARS
_____ Itchy ears
_____ Earaches/infections
_____ Drainage from ear(s)
_____ Ringing in ears, hearing loss
Total _____

EMOTIONS
_____ Mood swings
_____ Anxiety, fear or nervousness
_____ Anger, irritability or aggressiveness
_____ Depression
Total _____

ENERGY/ACTIVITY
_____ Fatigue, sluggishness
_____ Apathy, lethargy
_____ Hyperactivity
_____ Restlessness
Total _____

EYES
_____ Watery or itchy eyes
_____ Swollen, reddened or sticky lids
_____ Bags/dark circles
_____ Blurred/tunnel vision (not near- or far-sightedness)
Total _____

HEAD
_____ Headaches
_____ Faintness
_____ Dizziness
_____ Insomnia
Total _____

HEART
_____ Irregular/skipped heartbeat
_____ Rapid/pounding heartbeat
_____ Chest pain
Total _____

JOINTS/MUSCLES
_____ Pain/aches in joints
_____ Arthritis
_____ Stiffness or limited movement
_____ Pain/aches in muscles
_____ Feeling of weakness/tiredness
Total _____

LUNGS
_____ Chest congestion
_____ Asthma, bronchitis
_____ Shortness of breath
_____ Difficult breathing
Total _____

MIND
_____ Poor memory
_____ Confusion/poor comprehension
_____ Poor physical coordination
_____ Difficulty in making decisions
_____ Stuttering/stammering
_____ Slurred speech
_____ Learning disabilities
Total _____

MOUTH/THROAT
_____ Chronic coughing
_____ Gagging/frequent throat clearing
_____ Sore throat, hoarse/voice loss
_____ Swollen/discolored tongue, gum, lips
_____ Canker sores
Total _____

NOSE
_____ Stuffy nose
_____ Sinus problems
_____ Hay fever
_____ Sneezing attacks
_____ Excessive mucus formation
Total _____

SKIN
_____ Acne
_____ Hives, rash, or dry skin
_____ Hair loss
_____ Flushing/hot flashes
_____ Excessive sweating
Total _____

WEIGHT
_____ Binge eating/drinking
_____ Craving certain foods
_____ Excessive weight
_____ Compulsive eating
_____ Water retention
_____ Underweight
Total _____

OTHER
_____ Frequent illness
_____ Frequent/urgent urination
_____ Genital itch/discharge
Total _____

GRAND TOTAL _____

Figure 3. Immuno-Identity™ Questionnaire Scores Before and After Intervention

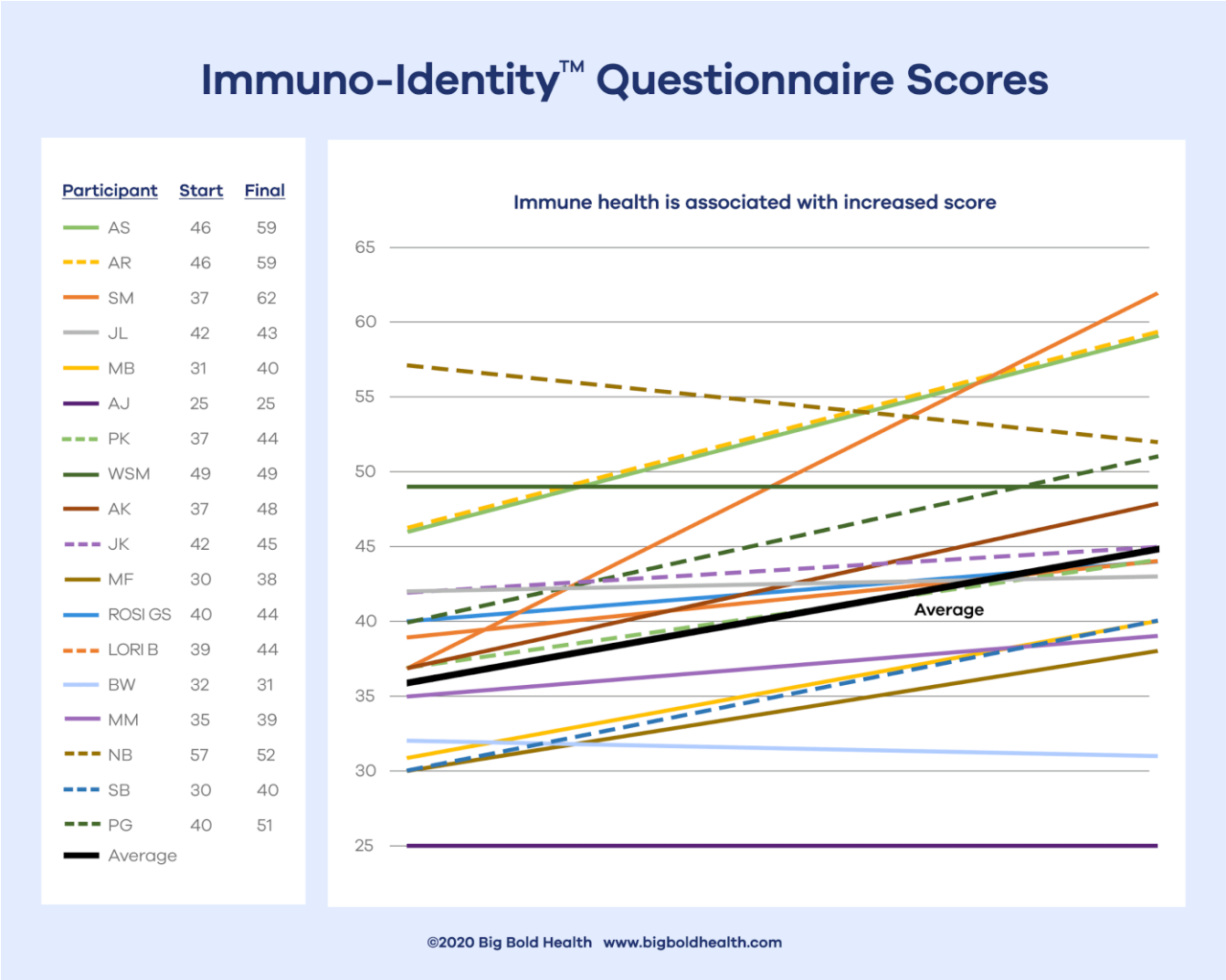
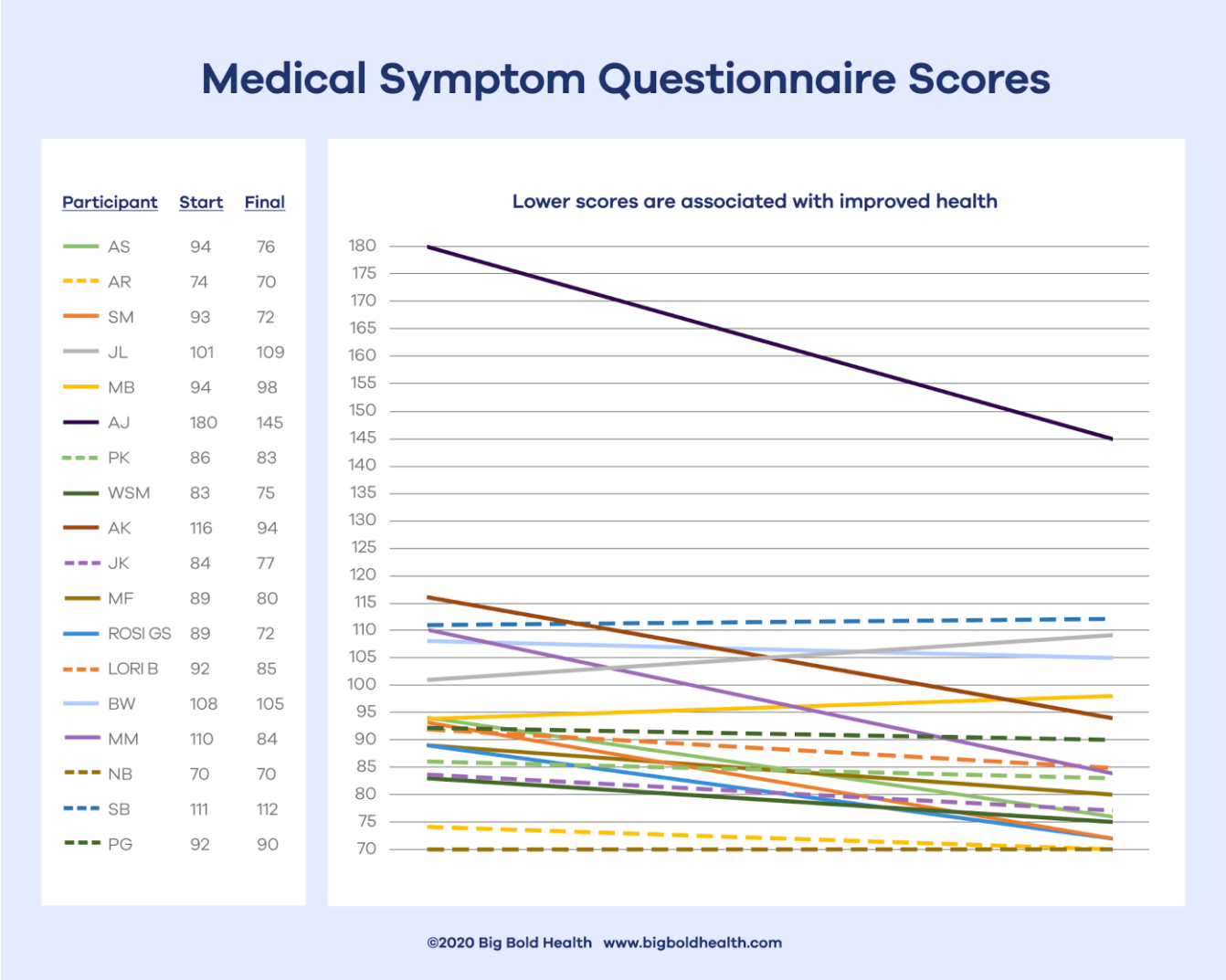


Figure 4. Medical Symptom Questionnaire Scores Before and After Intervention



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