THESIS

A CASE STUDY OF A WHEAT-FREE DIET ON AUTOIMMUNE DISEASE PROGRESSION

Submitted by

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In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Fall 2014

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ABSTRACT

A CASE STUDY OF A WHEAT-FREE DIET ON AUTOIMMUNE DISEASE PROGRESSION

Background and Aims: Autoimmune disease encompasses a broad range of over 80 conditions for which only three have an identified environmental trigger. Gliadin is the trigger in celiac disease, a condition that has been linked to other autoimmune conditions including Crohn's disease and type I diabetes (T1D). The purpose of this study was to investigate case studies of autoimmune patients who employed a wheat-free or Paleo-style diet (WFP) to manage their conditions.

Methods: A descriptive case study was performed that utilized questionnaires administered online and medical records from autoimmune disease patients who had consumed a WFP diet.

Results: Fifty-seven patients were evaluated in the study (mean age 37.3 yrs., SD 10.1), including 23 males and 34 females, 24 of whom provided medical records. Thirty of the 57 volunteers demonstrated signs of disease improvement while consuming a WFP. The rates varied across conditions with eight of eight Crohn's disease patients experiencing remission, while three of four T1D patients exhibited signs of improvement. Five of 15 patients with ankylosing spondylitis, rheumatoid arthritis, or undifferentiated and multiple connective tissue disorders worsened while on the diet.

Conclusions: Patients with single organ autoimmune diseases previously linked to increased intestinal permeability showed the greatest improvement after consuming a WFP. The results of

these case studies warrant further controlled research examining the effects of wheat consumption on Crohn's disease and T1D.

ACKNOWLEDGEMENTS

I wish to thank first my thesis committee for their constant understanding, patients and support. This research would not have happened without Dr. Cordain's wisdom, guidance, and knowledge. Dr. Hickey, despite constantly being overloaded, always made time and offered an ear when I needed it. Dr Ryan, who invited me into her lab, taught me a great deal about the research process that was always invaluable. Dr Schenkel provided invaluable knowledge and insight about immunology and was always supportive in my other life as a cyclist. I thank my wife who made too many sacrifices for me through this process and my parents who have been constantly supportive and encouraging in more ways than I can describe. Finally a special thanks to the Department of Health and Exercise Science and all of the faculty for helping me to pursue such an incredible area of study and allowing me to follow a unique path with my degree.

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CHAPTER I

INTRODUCTION

Autoimmune disease encompasses over 80 conditions whereby the immune system damages one or more of the body's own tissues (National Institutes of Health (U.S.), 2011). According to the U.S. National Institute of Health, between 14.7 to 23.5 million Americans (5 to 8% of the population) are affected (National Institutes of Health (U.S.), 2011). Autoimmune diseases share many common features and have both genetic and environmental components. Because incidence rates have been increasing, it is postulated that underlying environmental factors may be changing (The Autoimmune Diseases Coordinating Committee, 2005).

Organ-specific autoimmune conditions (ORACs), including Crohn's disease and type 1 diabetes (T1D), generally maintain one or a few auto-antibodies and damage a single organ or tissue. Crohn's disease causes inflammation of the gastrointestinal tract and can lead to a variety of complications including digestive and absorption issues, bleeding, and fistula formation (Lichtenstein, Hanauer, & Sandborn, 2009). T1D is characterized by the destruction of insulin-producing pancreatic β-cells leading to a life-long reliance on insulin injections (American Diabetes Association, 2014).

Systemic autoimmune conditions (SACs), including rheumatoid arthritis (RA) and mixed connective tissue disease (MCTD), affect multiple tissues and frequently maintain complex sets of auto-antibodies (Mahler & Fritzler, 2010). RA causes synovial inflammation and joint destruction throughout the body leading to chronic pain and disability (Scott, Wolfe, Huizinga, & Huizinga, 2010). MCTD shares characteristics with systemic lupus erythematosus, scleroderma,

and RA. It is identified by antibodies to extractable nuclear antigen (Sharp, Tan, Gould, & Holman, 1972).

The environmental triggers have been identified for only three autoimmune conditions: dermatitis herpetiformis; some variants of lupus; and celiac disease. Gliadin, the major constituent of gluten protein, bound to endogenous tissue transglutaminase is the environmental trigger in Celiac disease (Dieterich, et al., 1997). Celiac disease and the duration of exposure to gluten has been associated with multiple autoimmune conditions (Ventura, Magazzu, & Greco, 1999) including T1D (Smyth, et al., 2008), Crohn's disease (Tursi, Giorgetti, Brandimarte, & Elisei, 2005), and Hashimoto's thyroiditis (Counsell, Taha, & Ruddell, 1994) indicating a likelihood for common pathways. One potential pathway is a disruption of intestinal epithelial cell tight junctions which has been shown to precede celiac disease, T1D, and Crohn's pathogeneses (Bosi, et al., 2006; Drago, et al., 2006; Irvine & Marshall, 2000) leading some researchers to propose zonulin-mediated intestinal permeability as a possible regulator of autoimmune disease expression (Fasano, 2008). In both celiac and healthy subjects, gliadin in the intestinal lumen has been shown to upregulate zonulin and disrupt tight junctions (Visser, Rozing, Sapone, Lammers, & Fasano, 2009).

Research regarding wheat consumption and autoimmune progression outside of celiac disease is still limited. However, with the rising popularity of wheat-free diets like the Paleo Diet which promotes the consumption of lean meats, fruits, and vegetables (Cordain, et al., 2005), an increasing number of autoimmune patients have tried a wheat-free or Paleo-style diet (WFP) in hopes of better managing their symptoms. Varying degrees of success have been reported through blogs and media outlets. However, this self-assessed improvement generally was anecdotal and not supported by medical record analysis. The purpose of this descriptive case

study is to report on how a WFP diet may influence disease progression in autoimmune conditions.

CHAPTER II

METHODS

Study Design

A descriptive case-study was undertaken using medical records and questionnaire completed by study volunteers. The Institutional Review Board at Colorado State University approved the study. All participants provided electronic informed consent.

Study Participants

Adult volunteers were recruited online through autoimmune and Paleo diet discussion boards and blogs as well as directly from health care practitioners who have prescribed a WFP diet for autoimmune disease patients. To be eligible to participate, volunteers must have been diagnosed with a medically defined autoimmune condition at least six months prior to participation. Patient diagnoses were provided in their questionnaire answers and confirmed with their medical records when available.

To be included, participants had to be over 18 years of age, must have started a WFP diet after diagnosis and must have been on the diet for a minimum of two months. There was one exception with a participant who started a WFP several months after the onset of symptoms but still prior to official diagnosis. Participants were required to complete four questionnaires. Medical records detailing the history of their condition both prior to and after commencement of a WFP diet were requested but not required for inclusion. Participants were excluded if they did not complete all four questionnaires, if they communicated with the investigators in a way that jeopardized anonymity, or if no other volunteers had the same diagnosis.

Questionnaires

Participants completed a series of four online questionnaires using coded identification numbers to protect anonymity. Questionnaires included both quantitative and qualitative questions. The first questionnaire was prepared by the authors and addressed basic disease and diet history, participant demographics, and dietary compliance. Participants were asked to assess their disease progression since starting a WFP diet on an ordinal scale of + 3 to - 3 with a + 3 representing *remission* and a - 3 representing *significantly worse*. This first questionnaire served as informed consent. The second questionnaire, also prepared by the authors, contained questions expanding on disease, diet, and personal histories. Potential confounders such as medication, supplement use, other conditions, smoking behavior, physical activity, and environment were addressed.

The online version of the full Block 2005 food frequency questionnaire served as both the third and fourth questionnaires. It was administered online by Nutritionquest.com. Participants answered the questionnaire first to describe their diet prior to starting a WFP diet and then a second time to describe their diet while consuming a WFP diet. Multiple studies have demonstrated that this FFQ produces nutrient estimates similar to multiple day diet logs and can be valid for diet recalls up to 10-15 years (Sobell, Block, Koslowe, Tobin, & Andres, 1989).

Outcome Assessment

An assessment of whether patients improved, worsened, or did not change while following a WFP was conducted by the investigators independent of patient self-reported progression. The following criteria were used: a change in the biomarkers of their condition; a reported physical manifestation of change (i.e. hair regrowth, decreased lesions, increased pain,

etc.); a physician advised reduction or increase in medication; and an absence or increase in disease episodes. If a participant showed evidence of both improvement and worsening while eating a WFP diet, they were counted as worse.

Medical Records

Twenty-six participants provided records however two did not meet the inclusion criteria of the study. Participants were only asked to supply copies of their records to Colorado State University personnel directly. Records were considered *sufficient* if they provided enough endpoints to track participants' conditions both prior to and after starting a WFP diet. Of those 14 records, nine were considered *complete*, meaning they had enough endpoints to track the condition and the patient did not change their medication at approximately the same time that they started a WFP diet.

Statistics

Due to the descriptive nature of the study, only nonparametric percentages and means were used.

CHAPTER III

RESULTS AND CASE STUDIES

Participant Demographics

Ninety-four individuals were screened and consented for participation in the study. Sixty-nine completed all four questionnaires. Twenty-six provided medical records tracking their conditions but two were excluded from the study. After inclusion criteria were met, 57 subjects were evaluated representing the following conditions: Hashimoto's thyroiditis (n=18), Crohn's disease (n=8), psoriasis (n=8), ankylosing spondylitis (n=6), rheumatoid arthritis (n=5), multiple sclerosis (n=4), T1D (n=4), and undifferentiated or multiple connective tissue disorders (n=4). There were 34 (59.6%) females and 23 (40.4%) males. Mean age was 37.3 (SD ±10.1) years (range 21 to 62 years.) Table 1 provides full demographic data on all participants.

Table 1. Participant Demographics

All Participants	
Volunteered:	94
Completed All Four Questionnaires:	69
Met All Criteria:	57
Mean Age (SD):	37.3 (10.1)
Age Range:	21 to 62
Male:	23 of 57 (40.4%)
Female:	34 of 57 (59.6%)

Demographics by Condition

Condition	n	Provided Medical Records	Mean Age (SD)	Female	Male
All Organ-Specific	42	16	38.1 (10.4)	23 (54.8%)	19 (45.2%)
Crohn's disease	8	4	30.8 (8.1)	1 (12.5%)	7 (87.5%)
Type I diabetes	4	2	30.8 (4.5)	2 (50.0%)	2 (50.0%)
Hashimoto's Thyroiditis	18	8	41.6 (9.6)	14 (77.8%)	4 (22.2%)
Multiple sclerosis	4	1	38.0 (6.1)	3 (75.0%)	1 (25.0%)
Psoriasis	8	1	41.1 (13.6)	3 (37.5%)	5 (62.5%)
All Systemic	15	8	35.1 (9.1)	11 (73.3%)	4 (26.7%)
Ankylosing spondylitis	6	1	35.8 (6.6)	2 (33.3%)	4 (66.7%)
Rheumatoid arthritis	5	3	37.6 (14.0)	5 (100.0%)	0 (0.0%)
Connective tissue	4	4	30.8 (3.3)	4 (100.0%)	0 (0.0%)

Disease Progression While Consuming a WFP Diet

Results for the patient self-reported change in their condition are shown in Figure 1. The largest number of participants reported an improvement score of +2 while consuming a WFP. No participant self-reported that their condition worsened while on the diet.

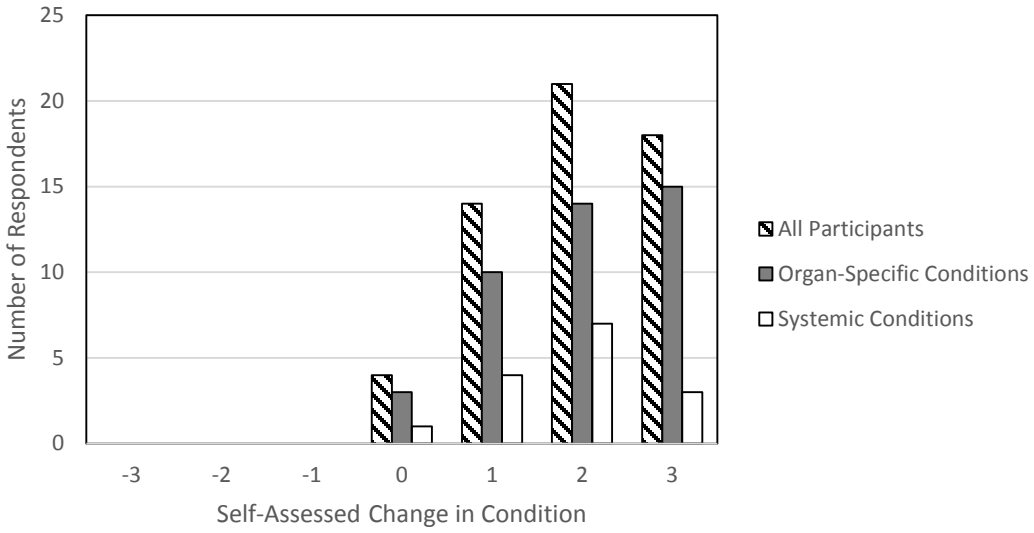


Figure 1. Participant self-assessed change in condition after starting a WFP diet based on an ordinal scale of +3 (remission) to -3 (significantly worse). A value of zero represented no change.

A separate investigator-assessed change in disease progression after starting a WFP diet is summarized in Table 2. Indications of improvement were highest among Crohn's disease patients at eight of eight and then among T1D participants at three of four, while ankylosing spondylitis, RA, and undifferentiated and multiple connective tissue disorders all had participants who appeared to worsen after starting the diet.

A novel finding of the study was a difference in the investigator-assessed rates of improvement between SACs and ORACs. Twenty-five of 42 (59.5%) participants with an ORAC demonstrated signs of improvement after starting a WFP diet compared to only five of 15

Table 2. Change in autoimmune symptom status after starting a WFP diet based on questionnaires and medical records

	n	Worsened on Diet		Improved on Diet		Remission on Diet†		Reduced Medication on Diet††	
		Total	%	Total	%	Total	%	Total	%
All Participants	57	5	8.8%	30	52.6%	10	15.8%	21	36.8%
All Organ-Specific	42	0	0.0%	25	59.5%	9	21.4%	15	35.7%
Crohn's	8	0	0.0%	8	100.0%	8	100.0%	7	87.5%
Diabetes	4	0	0.0%	3	75.0%	0	0.0%	2	50.0%
Hashimoto's	18	0	0.0%	8	44.4%	0	0.0%	4	22.2%
MS	4	0	0.0%	2	50.0%	1	25.0%	1	25.0%
Psoriasis	8	0	0.0%	4	50.0%	0	0.0%	1	12.5%
All Systemic	15	5	33.3%	5	33.3%	0	0.0%	6	40.0%
AS	6	2	33.3%	3	50.0%	0	0.0%	4	66.7%
RA	5	1	20.0%	2	40.0%	0	0.0%	2	40.0%
Connective	4	2	50.0%	0	0.0%	0	0.0%	0	0.0%

† based on current status listed in questionnaire or medical records when available.
†† refers only to a reduction in physician prescribed medication.

participants with an SAC. SAC patients were also more likely to experience a deterioration of their condition. 5 of 15 SAC participants worsened while following a WFP diet compared to no ORAC volunteers. Likewise, nine of 42 (21.4%) ORAC participants reported clinical remission after starting a WFP diet compared to no SAC participants.

Table 3. Medical Records Summary

Number of participants who provided medical records:	26 of 57
Number included in the study:	24 of 26
Records were sufficient:	14 of 24
Records were complete*:	9 of 14
Participants Who Improved While on WFP	
Number improved based on medical records deemed sufficient:	10 of 14 (71%)
Number improved based on medical records deemed complete:	7 of 9 (78%)
Participants Who Worsened While on WFP	
Number worsened while on WFP based on sufficient medical records:	3† of 14 (21%)

*Records were considered complete if they provided sufficient markers to track the patient's condition and the patient did not change medication at approximately the same time as starting a WFP diet.
†2 participants with MCTD and 1 participant with RA

Assessment of Medical Records

Participants who provided medical records are summarized in Table 3. A total of 26 records were received but one subject with sensory neuropathy and one subject with fibromyalgia did not meet the inclusion criteria. Nine subjects provided complete records. Medical improvement was demonstrated by seven of those nine after starting a WFP diet. Three participants provided records that indicated a worsening of their condition after starting a WFP diet but only two of those records were considered complete. The case studies below are limited to participants who provided sufficient medical records of their condition both prior to and while on a WFP.

Crohn's Disease Case Studies

Eight participants with Crohn's disease completed the questionnaires. Four provided medical records, three of which were considered complete. Of those three, all participants demonstrated medical signs of improvement. Eight of eight participants reported their status as clinical remission while on a WPF diet and seven of eight reduced or stopped taking medication.

Volunteer CD01, a 40-year-old male, was diagnosed with both Crohn's disease and Iron Deficiency in December of 2007. He started a wheat-free diet known as the Specific Carbohydrate diet in June of 2007 after experiencing symptoms which started in April of 2007. Unlike other participants, he started the diet prior to diagnosis. He was diagnosed with a melanoma at approximately the same time.

At the time of diagnosis, he had an elevated C-reactive protein (CRP) concentration of 9.2 mg/dl and colonoscopy results were described by his physician in the medical records as "significant ileitis and segmental colitis and EGD revealed severe erosive duodenitis with erosive esophagitis. Biopsies revealed granuloma within the duodenum and colon." He was placed on

metronidazole and budesonide at the time of diagnosis, but chose not to follow recommendations and stopped taking all medication a month after diagnosis.

In March of 2008, the physician noted in the medical records that “he has restricted himself from gluten containing products. He is now without abdominal pain, has gained 10 lbs., and feels well.” His physician goes on to write "I have indicated that current strategy of management by alternative approaches is not likely to control symptoms long term and it is highly likely he will experience a relapse of clinical symptoms. It is also likely he may develop other complications of Crohn’s disease.”

From 2008 to 2011, his CRP concentrations dropped to normal values (<1.0 mg/dl). Figure 2 shows the progression in CD01’s CRP concentrations. During an evaluation in 2010, his physician noted in the medical records that “laboratory 1/14/2010 revealed normal CBC, liver profile, C-reactive protein and fecal calprotectin.” The physician concluded that the “patient has high likelihood of Crohn’s disease and is in clinical remission. He is not on any maintenance therapy but remains on a dietary regimen that correlates with his improvement.” A follow-up visit was not recommended until 2012. The patient had blood work taken in January of 2011. CRP (<1.0 mg/dl) and calprotectin (21.1 mcg/g) were both normal. His hemoglobin levels had also normalized since the time of his diagnosis in 2009.

Volunteer CD02, a 26 year-old male, was diagnosed with Crohn’s disease in June of 2003. He described his condition at the time as follows: “I weighed ~137 lbs (6’3” frame). I had an active fistula as a result of perianal abscess which was lanced. Energy was very low, standing would result in faintness.” He started mesalamine in June of 2004. From 2005 to 2009, his condition was described as “active but mild.” In May of 2005, his colonoscopy report read “overall findings are consistent with active chronic inflammatory bowel disease, favoring

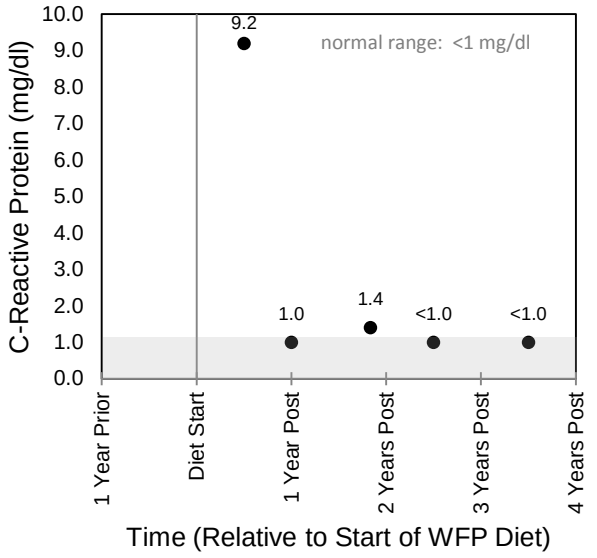


Figure 2. Change in C-Reactive Protein (CRP) for Crohn's Patient CD01.

Crohn's disease. However, with microscopic findings only, ulcerative colitis cannot be ruled out." In June of 2007, a further colonoscopy read "Crohn's ileocolitis with some minimal activity and large pseudopolyp. Rule out Neoplasia." There were multiple sites of granulation and inflammation. Finally, in June of 2008, colon and hepatic flexure showed "active chronic colitis with crypt architectural distortion consistent with chronic inflammatory bowel disease."

The patient started a WFP diet in July of 2009. A colonoscopy performed a month later in August of 2009 revealed "multiple puncate erosions" and "a large psuedopolyp at hepatic flexure." A biopsy demonstrated "no evidence of chronic or active colitis." The subject stopped taking medication two months later in October.

In July of 2010, one year after starting a WFP diet, tests revealed a "1 cm peduncilated polyp. Ileocecal valve, cecum, ascending hepatic flexure, splenic flexure, descending colon, sigmoid, and rectum were all in normal limits." A final colonoscopy in 2011 reported no activity. The patient's physician stated that yearly checkups were no longer necessary.

Type I Diabetes Case Studies

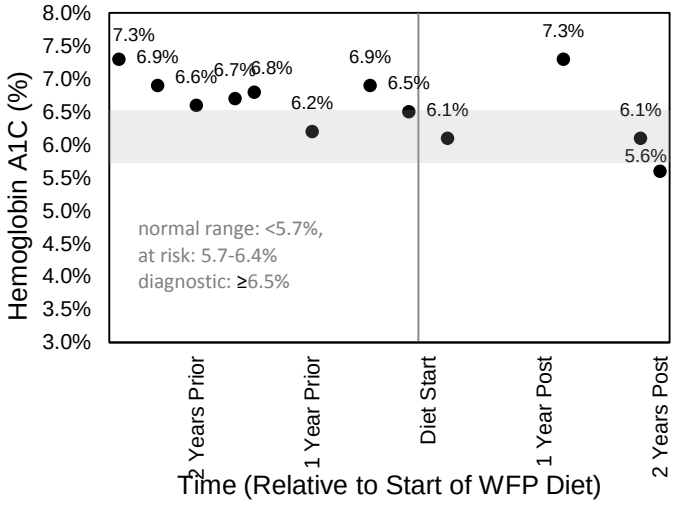
Four participants with T1D completed the questionnaires. Two participants provided medical records. Both showed signs of improvement but one started insulin aspart injections at the same time as the diet. Three of four exhibitied clear signs of improvement while eating a WFP diet. Two of four reduced medication while on the diet.

Participant DB01, a 31 year-old female, was diagnosed with T1D in 1989. She started taking insulin aspart and insulin glargine injections at the time of diagnosis and was still taking the medication at the time of this study. From 1989 until 2009, her A1C concentrations varied between 6.2% and 7.3% which is considered diagnostic of Diabetes according to American Dietetic Association guidelines (American Diabetes Association, 2014). Figure 3a shows the history of her A1C test results.

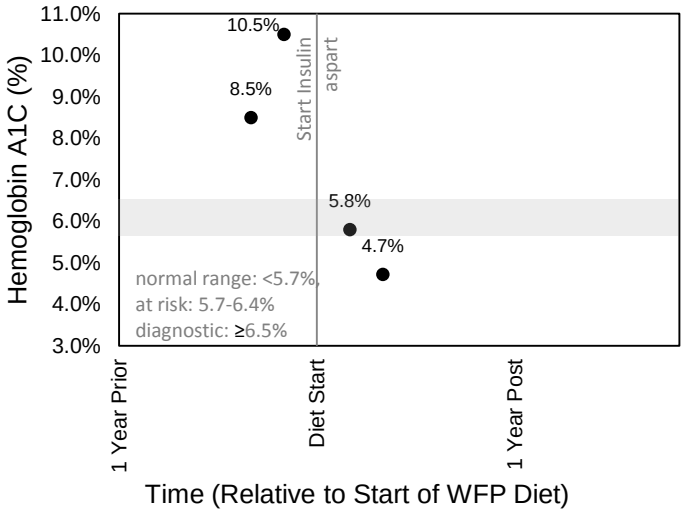
She started a WFP diet in March of 2009. Her A1C values generally dropped through February 2011 varying from 6.1% to a value of 5.6% which is considered below at-risk levels (American Diabetes Association, 2014). However, there was a high value of 7.3% one year after starting the diet. According the participant, she was able to reduce the dosage of her insulin injections soon after starting the diet. She described the change as follows: “My insulin sensitivity has increased so much, and I am still lowering my medications to this day. I do the Paleo Diet without fruit and it has been such a fantastic change. Still working to get my A1C below 5.0.” She commented that because she continues to alter her insulin dose “I do still experience some occasional highs and lows, though my blood sugars have improved immensely since changing my diet.”

Volunteer DB02, a 37 year-old female, experienced gestational diabetes in 2006 and 2008. In November of 2010 she was diagnosed with T1D. Two separate tests in November

produced A1C values of 8.5% and 10.5%. She started insulin aspart and insulin detemir injections at the time of diagnosis and began eating a WFP diet a month later in December of 2010. Figure 3b shows that her A1C values dropped to 5.8% in January of 2011 and 4.7% in April of 2011 which is well within normal levels (American Diabetes Association, 2014). In her 2011 medical records, her physician wrote "I do not think the patient is in the honeymoon phase."



a.



b.

Figure 3. Glycated hemoglobin (A1C) values for subjects (a) DB01 and (b) DB02. Subject DB02 started insulin aspart injections at the same time as a WFP diet. Ranges ares based on American Diabetes Association guidelines (American Diabetes Association, 2014).

Rheumatoid Arthritis Case Studies

Five volunteers with rheumatoid arthritis completed the questionnaires. Three provided medical records but only two were sufficient to track their conditions. Two of five participants showed signs of improvement while consuming a WFP diet and were able to reduce their medication, but only one supplied medical records. One subject clearly worsened while on the diet.

Participant RA01, a 26 year-old female, was diagnosed with a mild case of rheumatoid arthritis in February of 2009. At the time of diagnosis, her RF factor was 27 IU/ml and her Asparate Aminotransferase (AST) concentration was 41 U/L. However, her rheumatologist described her condition as follows: "Bone mineralization and alignment appear normal. Joint spaces are well preserved. There are no significant degenerative changes. There are no signs of inflammatory arthritis." Over the next two months she was placed first on methotrexate and then on etanercept.

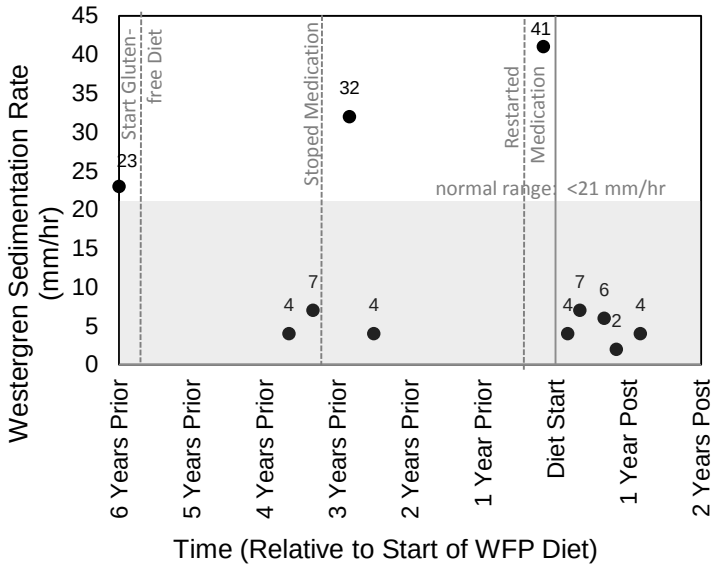
She started a WFP diet in October of 2009. By this time her AST concentrations had already returned to a normal value of 16 U/L and her first CRP test was also normal at 0.4 mg/dl. Five months later in March of 2010, her rheumatologist wrote in her medical records that the disease had halted: "The alignment, mineralization and joint spaces are normal. There are no erosive changes. There are no radiopaque foreign bodies or adnormal soft tissue calcifications" In April, she was switched from methotrexate and etanercept to hydroxychloroquine (a mild RA drug) and adalimumab. Through 2011, the patient was able to reduce the dosage of her medication and was considering going completely off medication at the time of this study.

Volunteer RA02, a 43 year-old female, had a complex history involving several changes in her diet and medication. In April of 2004, her Westergren sedimenation rate (WSR) was at 27

mm/hr which is considered high (Bottiger & Svedberg, 1967). Her CCP IgG antibody test was 242 units. Figure 4 shows the history of her WSR tests. She was diagnosed with seronegative rheumatoid arthritis and placed on methotrexate and hydroxychloroquine. She started a dairy/gluten-free diet in October of 2004. Two years later, in October of 2006, her rheumatologist described her condition in her medical records as follows: “She has been doing well on a reduced dose of methotrexate from 15 to 12.5 mg weekly… at this point she appears to be in a clinical remission state.” She continued to reduce her medication and by 2007, her rheumatologist stated “she has tolerated the reduction in dose well with no flare-ups.”

Late in 2007, she chose to stop medication despite physician warnings. She remained medication-free for over two years. During this time period, a WFP diet did not appear to be sufficient to manage her condition. Her rheumatologist described it as follows: "later she sought care of natural remedies on her own; saw homeopathic physician and eventually stopped all DMARDs for >2 years, but her RA flared and RA deformities/nodules quickly progressed and worsened during that time period."

In May of 2010, she restarted taking methotrexate. In July her CRP concentration was high at 15.4 mg/dl and her rheumatologist stated “her RA is very active and today we discussed at length the likely need for TNF like Enbrel.” She started etanercept (Enbrel) in July and started a full Paleo Diet in August of 2010. By September, her CRP was back down to 1.5 mg/dl and through 2011 there were no flares in her feet. Figure 4 shows the changes in her CRP concentrations. In November of 2011, her rheumatologist described her status as "multiple small joint synovitis, excessive morning stiffness and recurrent left knee effusions… improved a great deal after starting Enbrel."



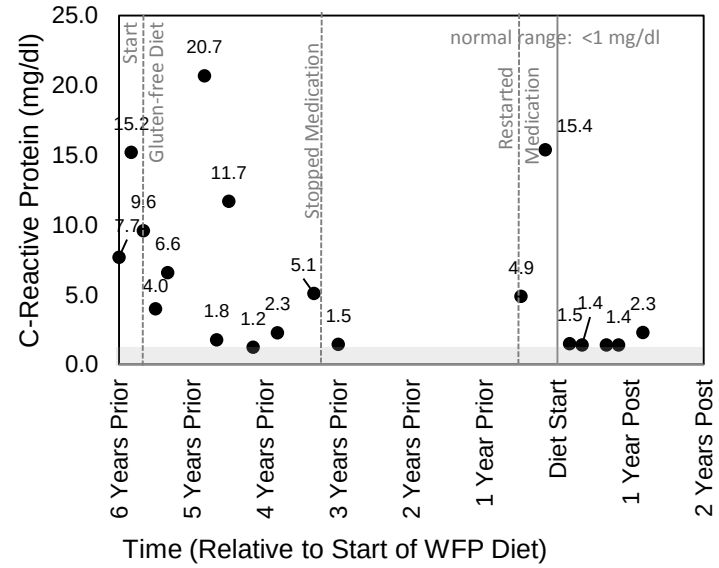


Figure 4. Westergren sedimentation rate and C-reactive protein values for subject RA02. Below 21 mm/hr is considered the normal Westergren Sedimentation rates for women under the age of 50 (Bottiger, et al., 1967).

Undifferentiated and Multiple Connective Tissue Disorders Case Studies

Four participants with undifferentiated or multiple connective tissues disorders completed the questionnaires. All four provided medical records of which two were considered sufficient. Those two both demonstrated some medical evidence of worsening while eating a WFP diet.

Volunteer MC01, a 31 year-old female, was diagnosed with MCTD in November of 2006. She started deltacortene in September of 2009, but remained on it for only two months. In October of 2009 and February of 2010, her Anti-Extractable Nuclear Antigen (ENA) screen, a test for MCTD (Sharp, et al., 1972), was positive. Figure 5 shows the changes in her compliment C3 concentrations which was normal at 141 mg/dl in February of 2010 (Health, 2010).

She started a WFP diet in March of 2010 and subsequently took deltacortene for another two months starting in June of 2010. Tests in November of 2010 maintained a positive ENA Screen. Her C3 concentrations dropped to 89 mg/dl which was borderline normal. Six months later in May of 2011 the patient's C3 concentration had dropped further to 86 mg/dl. Low C3 concentrations can indicate an autoimmune condition (Health, 2010).

In March of 2012, her diagnosis was changed to Systemic Lupus Erythematosus.

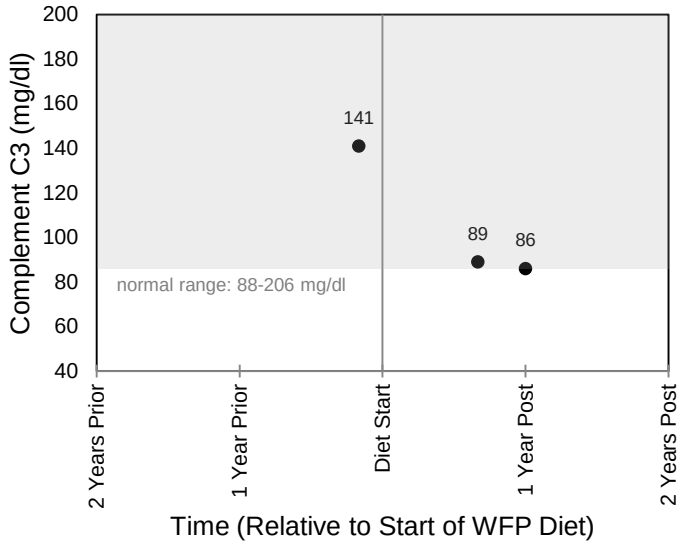


Figure 5. Complement C3 concentrations for subject MC01 just prior to start a WFP diet and for one year post. Normal complement C3 levels are based on NIH guidelines (Health, 2010).

Additional Case Studies

Additional case studies and conditions are provided in the Appendix.

CHAPTER IV

DISCUSSION

The primary finding of this limited study was that a WFP diet coincided with clinical improvement for some autoimmune disorders. Overall, 30 of 57 (52.6%) participants and seven of nine patients who provided complete medical records met the criteria for signs of improvement while eating a WFP diet. The rate of this investigator-assessed improvement varied across conditions. The highest rates of improvement were found in Crohn's disease and T1D patients. Eight of eight and three of four participants respectively demonstrated signs of improvement. On the other end of the spectrum was undifferentiated and multiple connective tissue diseases. No participants demonstrated improvement and two of four exhibited signs of worsening while on the diet.

All eight participants with Crohn's disease reported a state of remission while consuming a WFP diet and of those eight, seven stopped taking medication. It is important to note that Crohn's disease is an intermittent condition where patients naturally experience periods of remission (Russel, 2000). Increased intestinal permeability has been shown to proceed and even predict the course of Crohn's disease (Irvine, et al., 2000). Intestinal permeability can be influenced by gliadin from wheat (Drago, et al., 2006; Visser, et al., 2009) and Crohn's disease rates have been linked to celiac disease (Tursi, et al., 2005). Yet the investigators of this study are aware of only one study exploring the effects of wheat consumption on Crohn's pathogenesis. TNFARE/WT mice fed a gluten-fortified diet had an increased risk for Crohn's disease-like ileitis. The researchers identified gluten as an antigen-independent dietary factor (Wagner, Schmidt, Effenberger, Gruber, Danier, & Haller, 2013).

Improvement among T1D volunteers was determined primarily by a drop in A1C values and/or a prescribed reduction in medication. It is important to note that A1C values are a measure of glucose regulation and not a direct measure of disease pathology (American Diabetes Association, 2014). Due to the high glycemic load value of many wheat-based foods, a diet free of refined wheat products should aid glucose regulation (Wolever, Katzman-Relle, Jenkins, Vuksan, Josse, & Jenkins, 1994). Participants DM01 and DM04 both reported increased insulin sensitivity and reduced their medication dosages while consuming a WFP diet. As with Crohn's disease, intestinal permeability frequently precedes T1D (Bosi, et al., 2006; Mojibian, et al., 2009). However, unlike Crohn's disease, multiple studies have demonstrated a link between wheat consumption and T1D including a case study of a six-year-old boy who experienced remission without insulin therapy on a gluten-free diet (Mojibian, et al., 2009; Sildorf, Fredheim, Svensson, & Buschard, 2012).

A surprise finding of this study was the difference in improvement rates between SACs and ORACs. Twenty-five of 42 (59.5%) participants with an ORAC demonstrated signs of improvement while eating a WFP diet compared to five of 15 SAC participants. More importantly, an equal number of SAC participants, five of 15 exhibitied signs of worsening while on the diet. No one with an ORAC worsened while on the diet. Research on SACs and ORACs indicates potential differences in their mechanisms. Pathogenesis of ORACs reported on in this study have frequently been linked to an imbalance in T helper immune cells (Mesquita Jr, Cruvinel, Camara, Kallas, & Andrade, 2009). SACs, on the other hand, have been linked to a dysfunction in T Regulatory (T_{reg}) cells verses an imbalance (Longhi, et al., 2013; Miyara, Gorochov, Ehrenstein, Musset, Sakaguchi, & Amoura, 2011). Research on SACs has also pointed to DNA/RNA fragments, including small nuclear ribonucleoprotein particles (snRNPs),

as the autoantigens of many SACs (von Muhlen & Tan, 1995). Mammalian DNA is generally not immunogenic in healthy individuals indicating a potential defect in SACs (Stacey, et al., 2003). The investigators of this study are unaware of any studies linking a wheat-free diet or intestinal permeability to the immunogenicity of DNA, T_{reg} dysfunction, or to undifferentiated and multiple connective tissue diseases.

Due to the retrospective nature of this study, the primary limitation was a lack of control which included an inability to ensure that the participants were fully compliant with the WFP diet. As a result, no substantive conclusions could be drawn. However, the purpose of this study was not to establish a clear causal link between a WFP diet and autoimmune disease progression but instead, in a preliminary fashion, to identify potential links between the two.

Another limitation of the study was the selection process. Potential volunteers who had tried a WFP diet and experienced a negative response likely did not read the diet-related blogs and websites used for recruitment. As a result, it was assumed that the participant pool for this study was over-represented by those biased towards experiencing benefits from the diet. As expected, no participant self-reported worsening while they consumed a WFP diet. This bias was at least partially addressed by a separate investigator assessment of disease progression wherein five of 57 participants demonstrated signs of worsening and by limiting the case studies to participants who provided sufficient medical records.

In conclusion the patients with the autoimmune conditions reported on in this study exhibited different rates of improvement with the highest rate found in participants with organ-specific conditions that have been previously linked to increased intestinal permeability. The investigators felt that the case studies provide sufficient data to warrant further controlled studies

of the effects of a WFP diet on autoimmune conditions such as Crohn's disease and type 1 diabetes.

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APPENDIX A

ADDITIONAL RESULTS AND CASE STUDIES

Analysis of Dietary Change

The Full Block 2005 food frequency questionnaires (FFQ) was used to capture subjects' dietary behavior both while on the WFP diet and prior to the diet. Volunteers reported difficulty effectively capturing aspects of their WFP diet – meat consumption in particular. Participants provided feedback that the foods used in the FFQ, such as hamburgers and hot dogs, were based on a western diet and did not effectively capture the Paleo Diet food that many of them were eating such as lean grass-fed meats. Many ate food items that were not represented in the FFQ such as coconut milk and avocados. This is an issue that has already been identified in FFQ questionnaires (Ward, Keogh, Lentjes, Luben, Wareham, & Khaw, 2012). Due to this incompatibility with the WFP diet, it was likely that the FFQs underestimated calorie consumption and over-estimated fat consumption. The Block questionnaire has already been shown to overestimate fat consumption (Mares-Perlman, Klein, Klein, Ritter, Fisher, & Freudenheim, 1993).

Table 4 presents the primary mean differences between subjects' prior diet and WFP diet. Subjects self-assessed their compliance with the WFP diet at 91.4%. Mean compliance based on the FFQ did appear high. Average daily servings of bread, cereals, rice, and pasta dropped from 8.48 servings per day pre-diet to 0.23 servings while on a WFP diet. The FFQ did not differentiate wheat consumption specifically. While the focus of the study was on wheat consumption, mean daily servings of dairy products also dropped from 1.75 to 0.37 servings and vegetable consumption increased from 4.47 servings to 7.01 servings. Mean Glycemic Load

dropped significantly from 144.93 to 30.73. Changes for the SAC and ORAC subjects did not differ significantly from the mean changes for all subjects.

Table 4. Mean differences between diet prior to WFP and while consuming a WFP diet

	Prior to Wheat-Free/Paleo Diet	While On Wheat-Free/Paleo Diet
Self-Assessed Compliance with Diet		91.4%
Daily Calories (kcal)	**2816 kcal**	**1647.8 kcal***
Fat (%)	39.5%	55.13%*
Protein (%)	15.98%	20.96%*
CHO (%)	43.7%	22.87%*
Daily Glycemic Load	**144.93**	**30.73**
Daily Glycemic Index	49.45	40.31(Irvine, et al., 2000)
Calcium (mg/kcal as %)	44.2%	32.5%
Sodium:Potassium Ratio	1.23	1.12
Dietary fiber from fruit/veggies (g)	12.08g	16.11g
Dietary fiber from grains (g)	14.44g	3.32g
Daily Servings of Vegetables	4.47	7.01
Daily Frequency of fruits and fruit juices	1.63	1.19
Daily servings bread, cereals, rice, pasta	8.48	0.23
My Pyramid Grain - Total (1-oz equiv)	8.85	0.68
Daily Servings meat, fish, poultry, beans, eggs	3.76	3.81
My Pyramid Meat - fish, chix, meat (1 oz)	5.40	7.34
Daily Servings milk, yogurt, cheese	1.75	0.37
Beans	2-3 times per month	Never
Fat on Meat	Sometimes Ate It	Often Ate It
Fish	Once per month	Once per week
Bread	3-4 times per week	Never
Milk	Once per week	Never
Soda	Once per week	Never

* calorie consumption and fat/protein/CHO ratio may not be accurate for WFP diet due to incompatibility of the food frequency questionnaire with the typical WFP diet foods consumed by many of the participants.

Additional Crohn's Disease Case Studies

Patient CD03, a 23 year-old male, was diagnosed with Colitis in August of 2007. The participant did not provide colonoscopy results but did provide secondary blood markers. At the time of diagnosis, Mean Corpuscular Volume (MCV) at 73 fl and Mean Corpuscular Hemoglobin (MCH) at 22 ng were both just below normal ranges (Health, 2014). The patient

started azathioprine and xylazine in May of 2008. MCV (71 fl) and MCH (20 ng) were still low in August of 2008.

The volunteer started taking 10 mg/week of methotrexate and 100 mg of azathioprine in November of 2008 and was diagnosed with Crohn's disease in May of 2009. A blood test in September of 2009 revealed a very high C-Reactive Protein (CRP) level (34.01 mg/dl), but both MCV (89 fl) and MCH (31.2 ng) had normalized. Figure 2 shows CRP values for subject CD03.

In November of 2009 the patient started a WFP diet. Tests six months later in April of 2010 showed a normal CRP level (1.0 mg/dl). The subject stopped taking both azathioprine and xylazine in May of 2010 but remained on methotrexate and azathioprine. The most recent test in August of 2010 showed normal CRP (0.37 mg/dl), MCV (93.9 fl) and MCH (32.3 ng). The patient is currently in clinic remission. He described his status as "I am also on medication 100mg azathioprine and some sulfasalazine methotrexate 10 mg / week. I would like to give them up but I do not have the courage yet. Weakness is completely gone as the symptoms went away."

Additional Type I Diabetes Case Studies

Several other diabetes participants, who did not send medical records, also described improved glycemic control on a WFP diet that allowed them to reduce the dosage of their medications. Subject DM03, a 28 year-old male, provided a summary of his A1C values. Prior to the diet his values ranged between 7% and 8%. Several months after starting the diet, his first A1C test was 5.8% and his second was 6.2%. Another subject, DM04, a 27 year-old male commented that after going on the diet "I was much more sensitive to insulin, resulting in more frequent lows until I adjusted my dosages accordingly."

Hashimoto's Thyroiditis Case Studies

Eighteen participants with Hashimoto's thyroiditis completed the questionnaires. 8 of 18 (44.4%) had signs of improvement and 4 of 8 (22.2%) were able to reduce medication while on a WFP diet. Eight participants sent medical records, of which two were correlatable. Both of those participants had medical signs of improvement.

Patient HT01, a 49 year-old male, was diagnosed in March of 2010 and placed on T3 thyroid medication for Hashimoto's and human chorionic gonadotropin (HCG) and testosterone for adrenal fatigue. At the time of diagnosis several key markers of his condition were high including thyroid stimulating hormone (TSH) at 5.2 ulU/m, thyroglobulin antibodies (TgAb) at 1.3 U/m, and thyroglobulin at 25.0 ng/ml (Baloch, et al., 2003). Free T4 and free T3 were both within normal ranges. The patient's TSH concentrations normalized at 1.71 ulU/m two months after starting the T3 thyroid medication.

In November of 2010, he began a strict Paleo Diet, stopped the T3 thyroid medication, and started to reduce his testosterone and HCG medication. His TgAb concentrations had already normalized at <0.4 U/m when he started the diet which was likely due to the medication. By February of 2011 he was off all medication. Both TgAb and TSH concentrations remained normal throughout 2011 when he was managing his condition with diet alone. During the same period the patient's thyroglobulin concentrations steadily dropped from 22.2 ng/ml to a value of 6.8 ng/ml in September of 2011. Figure 6 traces the changes in TgAb and Thyroglobulin concentrations for volunteer HT01.

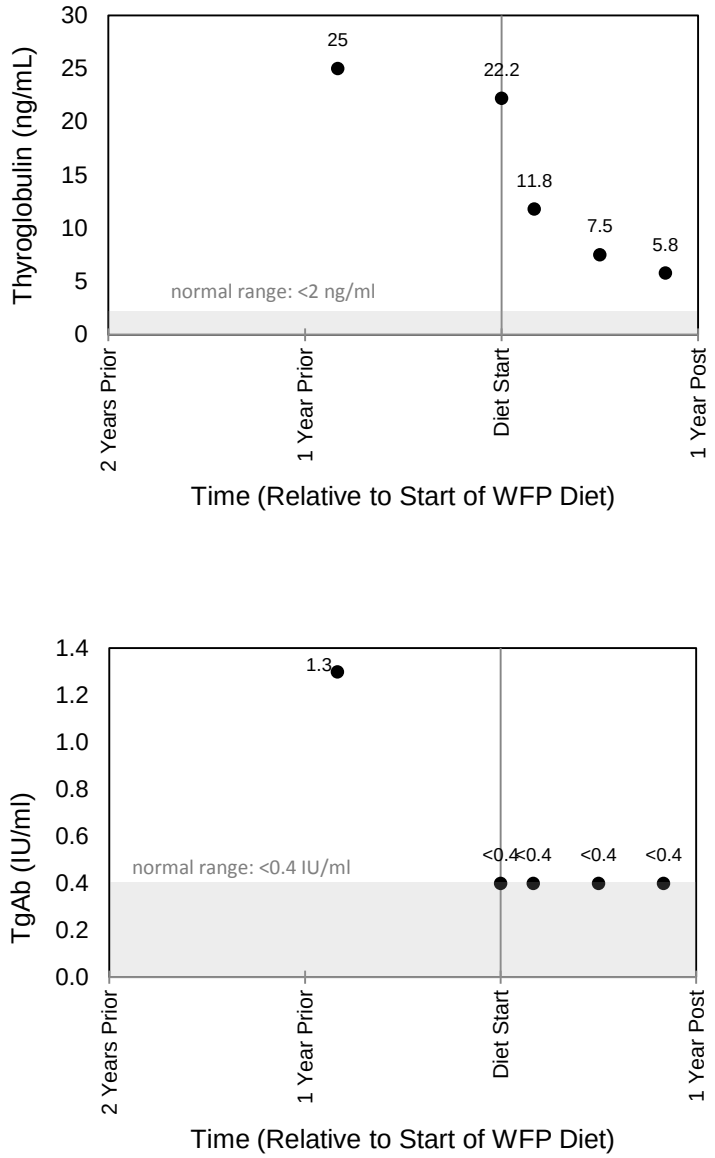

Figure 6. Thyroglobulin and TgAb levels for subject HT01. Tests did not differentiate TgAb levels below 0.4 IU/ml. Subject was diagnosed 8 months prior to diet and stopped all thyroid medication at the same time that he started a WFP diet.

Patient HT02, a 43 year-old female, was diagnosed with Hashimoto's thyroiditis in 1992. She took levothyroxine starting in 2002. Her condition was sub-clinical with no overt symptoms besides abnormal blood test results including a very high thyroid peroxidase antibody (TPOb) test at 120 U/ml in May of 2003 (Baloch, et al., 2003). Most tests conducted by her physician

evaluated TSH concentrations, a secondary marker of her condition. Figure 6 demonstrates that her TSH concentrations fluctuated between normal and high (2.23 to 8.08 ulU/m) in the three years prior to starting a WFP diet. In 2005, she started both a gluten and dairy-free diet. TSH concentrations normalized after starting the diet and remained in normal ranges (2.12 to 3.07 ulU/m). Interestingly the patient switched from levothyroxine to Armour Thyroid in January of 2010. Her TSH concentrations dropped from 2.9 to 0.7 ulU/m after starting the medication. In January of 2011 the patient was tested for gliadin intolerance. Her gliadin antibody IgG test was well within normal ranges at <3 U/ml. Volutneer HT02 reported that she did not consume wheat, diary, or fruit. The key finding in subject HT02 was a stabilization of her TSH concentrations after starting a WFP diet that did not correlate with any change in medication.

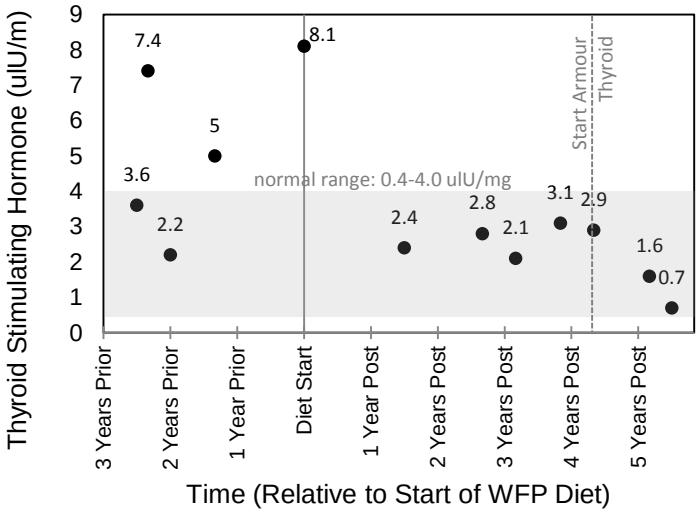

Figure 7. Thyroid stimulating hormone (TSH) concentrations for subject HT02. Normal range based on the National Academy of Clinical Biochemistry Guidelines (Baloch, et al., 2003).

Three Hashimoto's patients did not provide medical records but provided information on the markers of their condition in their questionnaires. Patient HT03, a 47 year-old female, had TSH concentrations which were high and fluctuated significantly between 5.0 and 69.9 ulU/m

prior to starting a WFP diet. On the diet, her concentrations normalized and her physician gradually reduced her medication (THYROID) from 180 mg to 60 mg per day. Volunteer HT04, a 61 year-old female, reduced her levothyroxine dose with physician recommendation from 125 μg to 75 μg per day in the nine months after starting a WFP diet. Volunteer HT05, a 48 year-old female, had her first negative TPOb test five months after starting a WFP diet.

Multiple Sclerosis, Ankylosing Spondylitis, and Psoriasis

The study included four participants with multiple sclerosis and one, patient MS01, sent medical records. While MS01's records showed improvement in her condition, the improvement started before she went on a WFP diet. Volunteer MS02 did not send medical records but claims that her physician has seen no observable progression and she has had no relapse since going on a WFP diet, while volunteer MS03 claims to be symptom free for the first time since diagnosis.

A total of six patients with ankylosing spondylitis, a systemic autoimmune condition, participated in the study. Only one participant, volunteer AS01, provided medical records which were not sufficient to track her condition. Further, she changed her medication at the same time that she started a WFP diet. Two of the participants, including patient AS01, reported that their symptoms continued to progress after going on a WFP diet. Volunteer AS01 had a normal Westergren sedimentation rate of 6 mm/hr three years prior to starting the diet. A second test a year and a half after the diet was on the border of normal at 25 mm/hr (Bottiger, et al., 1967). Volunteer AS02 did not provide medical records but she reported that she stopped all pain medication soon after going on a WFP diet in 2010. She claims to have had no flare-ups or pain in 2011. Participant AS03 reduced her medication from bi-weekly to monthly after starting a

WFP diet and eventually went off medication all together. Unfortunately she later had another large flare-up and returned to monthly medication.

Eight participants with psoriasis partook in the study and one sent medical records, but the records provided no markers relevant to his condition. Few of the participants took medication prior to or while on a WFP diet. Several participants reported reduction in lesions after starting the WFP diet. Patient PS01 reported that the guttate on his body went into remission and he no longer needed topical steroids. Volunteer PS02 claimed that her skin lesions had almost completely cleared since starting a WFP diet, but can reappear with greater severity when she eats wheat products.

Table 5. Summary of case study participants.

Subject	Sex	Condition	History		Key Changes in Medical Records	Status	Confounders
CD01	Male	Crohn's Disease	04.07 06.07 12.07	Start of symptoms Start of WFP diet Diagnosis Took Flagyl and Entocort for one month	• MCV (69 fL) and MCH (22.6 pg) were both low at start of diet but reached normal concentrations (88 fL and 30.4 pg) by 04.2009 • CRP (9.2 mg/dl) was high at time of diagnosis. Returned to normal (1.2 mg/dl) by 06.08	Subject has remained medication free and is in clinical remission. Regular follow ups no longer recommended	Subject started WFP diet prior to diagnosis
CD02	Male	Crohn's Disease	06.03 06.04 09.09 10.09	Diagnosis Start Pentasa Start of WFP diet Stopped medication	• Colonoscopies showed continued chronic colitis and periodic pseudopolyp development from 2005-2009 • 2011 colonoscopy - no sign of inflammation	In clinical remission and no longer needs regular checkups	
CD03	Male	Crohn's Disease	08.07 05.08 11.08 05.09 11.09 05.10	Colitis Diagnosis Start Imuran & xylazine Started methotrexate and azathioprine Crohn's Diagnosis Start WFP diet Stop Imuran & xylazine	• Colonoscopies were not provided • CRP concentrations (28-32 mg/dl) were high in 2008 to 2009. Normal CRP concentrations (0.37-1.0 mg/dl) in 2010 • Weight increased from 138 lbs to 155 lbs while on WFP diet	Subject is in clinical remission and has begun an exercise program	
DB01	Female	Type I Diabetes Mellitus	01.89 03.09	Diagnosis Start Novolog and Lantus Start of WFP diet	• A1C concentrations (varied 6.2% to 7.3%) remained high for four years prior to WFP diet • A1C concentrations dropped to normal range (5.6%) in 02.11	Continues to reduce medication while maintaining below diagnostic A1C concentrations	
DB02	Female	Type I Diabetes Mellitus	11.10 12.10	Diagnosis Start Novolog and Levemir Start WFP diet	• A1C concentrations were very high (10.5%) at time of diagnosis. Concentrations were back to normal (4.7%) by 04.11	A1C concentrations dropped from very high to normal values within five months of WFP diet start	Subject started WFP diet soon after medication
HT01	Male	Hashimoto's Thyroiditis	03.10 05.10 11.10 11.10	Diagnosis Start T3 thyroid and HCG medication Stopped medication Start WFP diet	• TgAb (1.3 U/mL), Thyroglobulin (25 ng/mL), and TSH (5.2 ulU/ml) were all above normal ranges 9 months prior to diet (02.10) • TSH normalized (1.71 ulU/ml) soon after starting T3 Thyroid and remained in normal ranges • TgAb concentrations normalized (<0.4) soon after starting WFP diet and remained normal • Thyroglobulin improved steadily from 22.2 ng/mL at start of diet to 6.8 ng/mL (09.11)	Stopped taking medication just prior to WFP diet. Primary immune markers of condition continued to improve for a year after the diet.	TSH and free T4 normalized prior to starting diet

HT02	Female	Hashimoto's Thyroiditis	1992 2002 09.05 01.10	Diagnosis Start levothyroxine Start WFP diet Stopped levothyroxine Start Armour Thyroid	• TSH concentrations fluctuated between normal and high (2.23-8.08 ulU/m) prior to WFP diet. Values remained normal (0.7-3.07 ulU/m) after 2005.	Condition was subclinical so no overt change in symptoms. TSH concentrations normalized on WFP diet
RA01	Female	Rheumatoid Arthritis	02.09 04.09 10.09 04.10	Diagnosis Start Methotrexate Start Enbrel Start WFP diet Switched to Plaquinel and Humira	• Rheumatologist reported a halt in progression five months after starting WFP (03.10) • MCV ranged 90-97 fL pre-diet and rose to slight above normal values (100-103 fL) while on diet	Condition was always mild but now no longer progressing. She is slowly reducing medication and hoping to try a drug-free remission
RA02	Female	Seronegative Rheumatoid Arthritis	04.04 07.04 10.04 2007 05.10 08.10	Diagnosis Start MTX and Plaquenil Start WFP diet Stopped medication Started methotrexate And Enbrel Started strict Paleo diet	• CRP concentrations were generally high (3.7 to 20.7 mg/dl) from 2004 through 2006. Concentrations were normal (1.4 to 4.9 mg/dl) while medication free but hit high concentrations again (15.4 mg/dl) during flare-up in 2010 • Sedimentation rate was very high (41 mm/hr) during flare-up in 2010 but returned to normal ranges (4 mm/hr) after starting Enbrel.	Subject had a very complex history. She attempted to manage condition with diet alone from 2007 to 2010. A flare-up of her condition in 2010 caused her to go back on medication that appeared to reduce inflammation.
MC01	Female	Mixed Connective Tissue Disorder	11.06 03.10 06.10 08.10 05.11	Diagnosis (approx.) Start WFP diet Start deltacortene Stopped medication Start Plaquenil	• Anti ENA screen was positive in all tests conducted between 10.09 and 11.10 • C3 (141 mg.dl) and C4 (20.6 mg/dl) were both normal just prior to diet. • C3 (86-89 mg.dl) dropped to below normal values in year after start of diet while C4 (13 mg/dl) became low normal	No medical evidence of improvement on WFP diet. Drop in C3 concentrations may indicate further progression.

APPENDIX B

INFORMED CONSENT FORM

Dear Participant,

My name is Trevor Connor and I am a researcher from Colorado State University in the Department of Health and Exercise Science. We are conducting a research study on the effects of a Paleo and/or wheat-free diet on autoimmune illness. The title of our project is the *Influence of a Paleolithic Diet on Autoimmune Progression*. The Principal Investigator is Dr. Loren Cordain, Health and Exercise Science and the Co-Principal Investigator is Trevor Connor, Health and Exercise Science.

We would like you to participate in a series of questionnaires related to your autoimmune condition and dietary behavior. Each questionnaire will take approximately 15 to 20 minutes to complete and you will be asked to complete between one and four questionnaires. Your participation in this research is voluntary. If you decide to participate in the study, you may withdraw your consent and stop participation at any time without penalty.

Each participant will be assigned an identification number in order to maintain the confidentiality of their answers and documents. A password protected electronic document will be maintained to determine which identification number is associated with which subject. This document will be the place where personal information (name, address, email address, phone number) about each subject will be maintained. The document will be kept on a local computer (to minimize the risk of unwarranted access.) Only the primary researchers in this study will have access to the document.

Questionnaires will be completed through an online password-protected website and only your identification number will be associated with the questionnaire. Completed questionnaires will be viewed by the Principle Investigators only.

We are also optionally asking for medical records related to your illness. These records are voluntary and not required for participation, but will aid the study significantly. Specifically, we will look at changes in the specific symptoms and markers related to your disease (i.e. blood markers, neurological tests, etc.) When the medical records are received, they will be scanned and any information that can associate the documents with the subject (i.e. name, address, phone number) will be erased and replaced with the subject's identification number. Once scanned, the original documents will be kept in a secure, locked cabinet maintained in the department of Health and Exercise at Colorado State University.

While there are no direct benefits to you, we hope to gain more knowledge on how a Paleolithic/wheat-free diet may help sufferers of autoimmune conditions. Participation in this study presents no direct risk to the participant. It is not possible to identify all potential risks in research procedures, but the researcher(s) have taken reasonable safeguards to minimize any known and potential, but unknown, risks.

If you have any questions, please contact Trevor Connor at [redacted] or [redacted]. If you have any questions about your rights as a volunteer in this research, contact Janell Barker, Human Research Administrator, at [redacted].

Sincerely,

Loren Cordain	Trevor Connor
Professor, Health and Exercise Science	Graduate Student, Health and Exercise Science

APPENDIX C

FIRST INVESTIGATOR-CREATED QUESTIONNAIRE

DEPARTMENT OF HEALTH AND EXERCISE SCIENCE
COLORADO STATE UNIVERSITY
DIET AND AUTOIMMUNITY PRELIMINARY QUESTIONNAIRE

STUDY_____ **DATE**_____ **SUBJECT ID #**_____

Reviewed by:_____

THE FOLLOWING QUESTIONNAIRE SHOULD TAKE APPROXIMATELY 10 MINUTES TO COMPLETE. PLEASE ANSWER ALL QUESTIONS TO THE BEST OF YOUR KNOWLEDGE AND PRINT CLEARLY.

SECTION I: DEMOGRAPHIC INFORMATION

Age _____ Date of Birth (MM/YYYY) _____

Sex _____

Height _____

Current Weight _____

Ethnic Background:

- ☐ Black or African American
- ☐ Asian
- ☐ Native Hawaiian or Other Pacific Islander
- ☐ White or Caucasian
- ☐ Native American or Alaska Native
- ☐ Hispanic or Latino or Spanish Origin
- ☐ White, non-Hispanic
- ☐ Unknown
- ☐ Decline to Report

SECTION II: AUTOIMMUNE DISEASES AND OTHER DISEASES THAT MAY HAVE AN AUTOIMMINE COMPONENT

What conditions have you been diagnosed with?

		Date of Diagnosis (month/year)	*Current Status*
Alopecia Areata	☐		
Ankylosing Spondylitis	☐		
Ataxia	☐		
Crohn's Disease	☐		
Grave's Disease	☐		
Huntington's Disease	☐		

Hashimoto's Thyroiditis	☐	______________________________
Irritable Bowel	☐	______________________________
Kawasaki's Disease	☐	______________________________
Lupus Nephritis	☐	______________________________
Lupus Erythematous	☐	______________________________
Multiple Sclerosis	☐	______________________________
Psoriasis	☐	______________________________
Rheumatoid Arthritis	☐	______________________________
Type I Diabetes Mellitus	☐	______________________________
Ulcerative Colitis	☐	______________________________
Uveitis	☐	______________________________
Vitiligo	☐	______________________________
Other______________________	☐	______________________________

(Other Potential Illnesses Include: Antiphospholipid Syndrome, Addison's Disease, Hemolytic Anemia, Meniers Disease, Goodpasture's Syndrome, Lymphoproliferative Syndrome, Myasthenia Gravis, Oophoritis, Becet's Disease, Bullous Pemphigoid, Dermatomyositis, Glomerulonephritis, Gullain-Barre Syndrome, Sarcoidosis, Sjogren's Syndrome, Pemphigus/Pemphigoid, Polyarteritis Nodosa, Wegener's Granulomatosis)

SYMPTOMOLOGY

Approximately when did you first notice symptoms (MM/YYYY): ________________

Please list all symptoms that you have experienced as a result of the illness:

	Severity
______________________________	(very severe) 1 2 3 4 5 6 7 (very mild)
______________________________	(very severe) 1 2 3 4 5 6 7 (very mild)
______________________________	(very severe) 1 2 3 4 5 6 7 (very mild)
______________________________	(very severe) 1 2 3 4 5 6 7 (very mild)
______________________________	(very severe) 1 2 3 4 5 6 7 (very mild)
______________________________	(very severe) 1 2 3 4 5 6 7 (very mild)
______________________________	(very severe) 1 2 3 4 5 6 7 (very mild)
______________________________	(very severe) 1 2 3 4 5 6 7 (very mild)

SECTION III: DIET HISTORY

When did you start a Paleo/wheat-free diet (MM/YYYY)? ________________________

Are you still on the diet? YES ☐ NO ☐ If No, please explain why you stopped:

How long have you been/were you on the diet (months and years)? _____

While on the diet, how many times per week did/do you eat the following foods?

Sausage_____	Bacon_____	Beef_____	Poultry_____
Pork_____	Cheese_____	Fish_____	Eggs_____
Shellfish_____	Fried Foods_____	Breads_____	Cereals_____
Fruits_____	Vegetables_____	Desserts_____	Soybeans_____
Tomatoes_____	Bananas _____	Potatoes_____	Wheat_____
Quinoa_____	Alfalfa Sprout_____	Beans_____	Peanuts_____

Jalapeno Peppers _____ Spicy Peppers _____ Hot Sauce _____

Other_____ (describe)

How many servings per week of the following did you normally consume:

Whole milk_____	2% Milk_____	Skim milk_____	Buttermilk_____
Coffee_____	Tea_____	Soft-Drinks_____	Beer_____
Wine_____	Liquor_____	Water_____	Root Beer_____

If you consume eggs or meat, would you say you primarily eat (check all that apply):

Eggs: ☐ Omega-3 Eggs ☐ From free-range chickens

Meats: ☐ Grass-fed ☐ Low-Fat (99% lean by weight) ☐ High Fat (95% lean or less by weight)

Please rate how compliant you are/were with your Paleo/wheat-free diet: _____
(0% is not at all while 100% is completely compliant)

Would you describe your compliance as:

(Break the "rules" frequently) 1 2 3 4 5 6 7 (Never break the rules)

(Periodic or On-and-Off) 1 2 3 4 5 6 7 (Constant)

Have you ever been a vegetarian? Yes No
If so, approximately what dates (give months and years): started _____ stopped _____

SECTION IV: DIET AND DISEASE STATUS

Have any symptoms improved or gotten worse since starting a Paleo/wheat-free diet (please list and describe)?

Symptom	*Change*
_____	(much worse) -3 -2 -1 0 1 2 3 (completely gone)
_____	(much worse) -3 -2 -1 0 1 2 3 (completely gone)
_____	(much worse) -3 -2 -1 0 1 2 3 (completely gone)
_____	(much worse) -3 -2 -1 0 1 2 3 (completely gone)
_____	(much worse) -3 -2 -1 0 1 2 3 (completely gone)
_____	(much worse) -3 -2 -1 0 1 2 3 (completely gone)
_____	(much worse) -3 -2 -1 0 1 2 3 (completely gone)
_____	(much worse) -3 -2 -1 0 1 2 3 (completely gone)

Description:

If you or your doctor has witnessed any measureable changes in your blood or other markers (such as weight, thyroid hormone, cholesterol, or any markers related to your condition,) please list them below:

Biomarker	*Prior to Diet*	*After Diet*
Blood Pressure	Systolic/Diastolic: _____	Systolic/Diastolic:_____
Cholesterol	Total:_____ HDL:_____ LDL:_____	Total:_____ HDL:_____ LDL:_____
Body Weight	_____	_____
Triglyceride Levels	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

How would you describe the change in your overall disease progression since starting a Paleo/wheat-free diet:

Overall:		(significantly worse) -3 -2 -1 0 1 2 3 (remission)
Prior to the Diet:	N/A	(significantly worse) -3 -2 -1 0 1 2 3 (remission)
1 week after starting the diet:	N/A	(significantly worse) -3 -2 -1 0 1 2 3 (remission)
1 month after starting the diet:	N/A	(significantly worse) -3 -2 -1 0 1 2 3 (remission)
6 months after starting the diet:	N/A	(significantly worse) -3 -2 -1 0 1 2 3 (remission)
1 year after starting the diet:	N/A	(significantly worse) -3 -2 -1 0 1 2 3 (remission)

SECTION V: ADDITIONAL INFORMATION

SECOND QUESTIONNAIRE

Would you be willing to participate in the second part of this questionnaire process? It would include a second questionnaire of approximately 15 minutes asking further details about your condition (such as medication,) diet, and environmental factors (such as where you live and physical activity.) In addition, you would be asked to complete two standardized food questionnaires about your eating habits. Each would take 15 to 20 minutes and can be completed at separate times.

YES ☐ NO ☐

MEDICAL RECORDS

Are you willing to provide your medical records from just prior to starting the diet and your most recent records? This is strictly voluntary. You would provide us with your records directly and would provide only those records you are comfortable sharing. We would make no attempt to contact your doctor and you will not be required to sign any release. Any records you send will be kept strictly confidential.

YES ☐ NO ☐

APPENDIX D

SECOND INVESTIGATOR-CREATED QUESTIONNAIRE

DEPARTMENT OF HEALTH AND EXERCISE SCIENCE
COLORADO STATE UNIVERSITY
DIET AND AUTOIMMUNITY FINAL QUESTIONNAIRE

STUDY_____ **DATE**_____ **SUBJECT ID #**_____

Reviewed by:_____

THE FOLLOWING QUESTIONNAIRE SHOULD TAKE APPROXIMATELY 15-20 MINUTES TO COMPLETE. IN addition YOU WILL BE ASKED TO COMPLETE TWO FOOD FREQUENCY QUESTIONNAIRES PROVIDED ON A SEPARATE WEBSITE. PLEASE ANSWER ALL QUESTIONS TO THE BEST OF YOUR KNOWLEDGE.

SECTION I: ADDITIONAL AUTOIMMUNE ILLNESS HISTORY

Is there a history of similar autoimmune illness in your family?

	Diagnosis	Age of Onset
Father		
Mother		
Brothers/Sisters		

MEDICATION

Do you regularly take any painkillers since being diagnosed:

Pain killer	*Taken While On Paleo/ Wheat-free Diet*		*Frequency*								
Ibuprofen	YES ☐	NO ☐	(daily)	1	2	3	4	5	6	7	(rarely)
Aspirin	YES ☐	NO ☐	(daily)	1	2	3	4	5	6	7	(rarely)
_____	YES ☐	NO ☐	(daily)	1	2	3	4	5	6	7	(rarely)
_____	YES ☐	NO ☐	(daily)	1	2	3	4	5	6	7	(rarely)

Are you on or have you taken any medication for your condition?

Medication	*Start Date (mm/yyyy)*	*End Date (or current)*	*Taken While On Paleo/ Wheat-free Diet*	
Corticosteroid Steroids________	________	________	YES ☐	NO ☐
________	________	________	YES ☐	NO ☐
________	________	________	YES ☐	NO ☐
________	________	________	YES ☐	NO ☐
________	________	________	YES ☐	NO ☐
________	________	________	YES ☐	NO ☐

In general, how compliant have you been with your medication?

Medication	*Compliance*
________	(never take) 1 2 3 4 5 6 7 (always take)
________	(never take) 1 2 3 4 5 6 7 (always take)
________	(never take) 1 2 3 4 5 6 7 (always take)
________	(never take) 1 2 3 4 5 6 7 (always take)
________	(never take) 1 2 3 4 5 6 7 (always take)

Have any symptoms improved or disappeared since starting the medication (please list and rate)?

Symptom	*Change*
________	(much worse) -3 -2 -1 0 1 2 3 (completely gone)
________	(much worse) -3 -2 -1 0 1 2 3 (completely gone)
________	(much worse) -3 -2 -1 0 1 2 3 (completely gone)
________	(much worse) -3 -2 -1 0 1 2 3 (completely gone)
________	(much worse) -3 -2 -1 0 1 2 3 (completely gone)
________	(much worse) -3 -2 -1 0 1 2 3 (completely gone)
________	(much worse) -3 -2 -1 0 1 2 3 (completely gone)
________	(much worse) -3 -2 -1 0 1 2 3 (completely gone)
________	(much worse) -3 -2 -1 0 1 2 3 (completely gone)

SECTION II: DIET HISTORY

PALEO/WHEAT-FREE DIET

Are there any foods you reduced or removed from your diet after starting a Paleo/wheat-free diet?

Food

_____ (reduced slightly) 1 2 3 4 5 6 7 (completely removed)
_____ (reduced slightly) 1 2 3 4 5 6 7 (completely removed)
_____ (reduced slightly) 1 2 3 4 5 6 7 (completely removed)
_____ (reduced slightly) 1 2 3 4 5 6 7 (completely removed)
_____ (reduced slightly) 1 2 3 4 5 6 7 (completely removed)

Are there any foods you increased in your diet after starting a Paleo/wheat-free diet?

Food

_____ (increased slightly) 1 2 3 4 5 6 7 (significant increase)
_____ (increased slightly) 1 2 3 4 5 6 7 (significant increase)
_____ (increased slightly) 1 2 3 4 5 6 7 (significant increase)
_____ (increased slightly) 1 2 3 4 5 6 7 (significant increase)
_____ (increased slightly) 1 2 3 4 5 6 7 (significant increase)

Please describe any other changes you made to your diet after switched to a Paleo/wheat-free diet not covered above:

SUPPLEMENTS

Please list any supplements you have taken while on the Paleo/wheat-free diet or up to 1 year before starting the diet:

Supplement	*Dose*	*Start Date (mm/yyyy)*	*End Date (or current)*	*Taken While On Paleo/ Wheat-free Diet*
n-3 Fish Oils	_____	_____	_____	YES ☐ NO ☐
Flax Oil	_____	_____	_____	YES ☐ NO ☐
Vitamin D	_____	_____	_____	YES ☐ NO ☐
Probiotics	_____	_____	_____	YES ☐ NO ☐
Prebiotics (cilium)	_____	_____	_____	YES ☐ NO ☐
_____	_____	_____	_____	YES ☐ NO ☐
_____	_____	_____	_____	YES ☐ NO ☐
_____	_____	_____	_____	YES ☐ NO ☐
_____	_____	_____	_____	YES ☐ NO ☐

SECTION III: ENVIRONMENT AND LIFESTYLE FACTORS

Please list the locations you've lived since one year prior to your diagnosis:

City *State/Province* *Country* *Move In Date* *Move Out Date*

_____

_____

_____

_____

_____

_____

OCCUPATION AND STRESS LEVELS

Please list your occupations starting from one year prior to your diagnosis

Occupation	*Start Date*	*End Date*	*Primarily* *Indoor*	*Outdoor*
_____			☐	☐
_____			☐	☐
_____			☐	☐
_____			☐	☐
_____			☐	☐
_____			☐	☐

Please describe your average stress levels:

Prior to Diagnosis: (high) 1 2 3 4 5 6 7 (low)
Post Diagnosis: (high) 1 2 3 4 5 6 7 (low)

PHYSICAL ACTIVITY

How would you describe your level of activity:

Prior to Diagnosis: (no activity) 1 2 3 4 5 6 7 (highly active)
Approximate hours/week: _____

After Diagnosis: (no activity) 1 2 3 4 5 6 7 (highly active)
Approximate hours/week: _____

After starting Paleo/wheat-free diet: (no activity) 1 2 3 4 5 6 7 (highly active)
Approximate hours/week: _____

Currently: (no activity) 1 2 3 4 5 6 7 (highly active)
Approximate hours/week: _____

Please list any exercises/physical activity that you engage in on a regular basis (i.e. running, yoga, cycling, yard work):

TOBACCO HISTORY (check one)

None ☐
Quit ☐ (when)_____
Cigarette ☐
Cigar ☐
Pipe ☐
Chew Tobacco ☐
Snuff ☐
Total years of tobacco use_____

CURRENT TOBACCO USE
(if applicable)

	# per day
Cigarette	_____
Cigar	_____
Pipe	_____
Chew Tobacco	_____
Snuff	_____

SECTION IV: OTHER MEDICAL FACTORS

BODY WEIGHT

What was your approximate weight:

Just prior to diagnosis _____
Just prior to starting the diet _____
At the end of the diet (if applicable) _____
Currently _____

OTHER CONDITIONS AND MEDICATIONS

Since being diagnosed, have you regularly taken any of the following:

	Taken While On Paleo/ Wheat-free Diet		*Frequency*	
Contraceptive Medication:	YES ☐	NO ☐	after diagnosis:	(daily) 1 2 3 4 5 6 7 (rarely)
(please list)_____			while on diet:	(daily) 1 2 3 4 5 6 7 (rarely)
Antacids	YES ☐	NO ☐	after diagnosis:	(daily) 1 2 3 4 5 6 7 (rarely)
(please list)_____			while on diet:	(daily) 1 2 3 4 5 6 7 (rarely)
Medical Marijuana	YES ☐	NO ☐	after diagnosis:	(daily) 1 2 3 4 5 6 7 (rarely)
			while on diet:	(daily) 1 2 3 4 5 6 7 (rarely)

Have you been diagnosed with or treated for any other conditions (check all that apply):

		Date of Diagnosis (MM/YYYY)	*Details (optional)*
High blood pressure	☐	_____	_____
Heart Attack	☐	_____	_____
Stroke	☐	_____	_____
Cancer	☐	_____	_____
High Cholesterol	☐	_____	_____
_____	☐	_____	_____
_____	☐	_____	_____
_____	☐	_____	_____

Did/do you take medication for any of the above conditions:

Medication	*Start Date (mm/yyyy)*	*End Date (or current)*	*Taken While On Paleo/ Wheat-free Diet*
_____	_____	_____	YES ☐ NO ☐
_____	_____	_____	YES ☐ NO ☐
_____	_____	_____	YES ☐ NO ☐
_____	_____	_____	YES ☐ NO ☐
_____	_____	_____	YES ☐ NO ☐

Have you ever been hospitalized or had surgery? YES ☐ NO ☐
If Yes, please explain: (include date and type of surgery, if possible)

INFECTIOUS DISEASE AND VACCINE HISTORY

Have you been diagnosed with any of the following viral infections:

Coxsackie B	☐
Hepatitis B or C	☐
Epstein-Barr	☐
Human Parvovirus B19	☐

Have you been vaccinated for any of the following:

Vaccination	*Age*	
Diphtheria	_____	☐
Tetanus	_____	☐
Polio	_____	☐
Measles	_____	☐
Hepatitis B	_____	☐

Have you ever been treated with radiation YES ☐ NO ☐ Year: _____
Have you suffered from any chronic infections YES ☐ NO ☐ Infection: _____

APPENDIX E

DISCLAIMER

DEPARTMENT OF HEALTH AND EXERCISE SCIENCE
COLORADO STATE UNIVERSITY
DIET AND AUTOIMMUNITY STANDARD COMMUNICATIONS

The following is a disclaimer that will be included on both the questionnaires and all critical communications with study subjects:

Disclaimer

This study is in no way affiliated with, sponsored, or endorsed by any third party interests such as pharmaceutical or food companies. This study is not designed to be diagnostic or provide medical advice for the subjects who participate. This study is designed for information gathering purposes only. Participation in this study is entirely voluntary. Volunteers enter this study willingly and with no expectation of compensation, answers, or solutions. All participants are advised to fully read the consent form before participating. Any medical questions should be referred to the participant's physician before participating in this study.

APPENDIX F

STANDARD COMMUNICATIONS

DEPARTMENT OF HEALTH AND EXERCISE SCIENCE
COLORADO STATE UNIVERSITY
DIET AND AUTOIMMUNITY STANDARD COMMUNICATIONS

Below are templates that will be used to handle communications with subjects in the initial subject identification step of this study:

Initial Communication with Discussion Board Owners

My Name is Trevor Connor. I'm a Master's student working with Dr Loren Cordain at Colorado State University. Dr Cordain and I are currently researching potential dietary triggers of autoimmune illness. In particular we are looking at the effects of a Paleo style diet and whether there are any common trends across autoimmune diseases.

As part of our research, we'd like to conduct a cross-sectional study of people who have autoimmune illness and have tried a Paleo style or wheat-free diet. For now, we are planning an initial informational study. It would involve a series of questionnaires with volunteers to assess whether the diet had any noticeable effect (positive or negative) on their illness and to try to identify any potential trends in their dietary behavior and disease progression. We would of course follow all human subject ethical guidelines and information would be kept secure and confidential through the use of unique identifiers for each subject (their names will not be used.)

With your permission, I'd like to create a post on your forum asking for volunteers. A copy of the message I was planning on posting is included at the bottom of this email. If you are willing, please let me know if there is a particular section of the forum where you'd like me to post this call for volunteers. Of course, if you have any questions, don't hesitate to contact me. My email address is [redacted]. Either way, I will wait to hear back from you.

Thank you,
Trevor Connor
Colorado State University

Discussion Board Post

Title: Seeking Volunteers With Autoimmune Illness Who Have Tried a Paleo/Wheat Free Diet…

Hello forum subscribers. I am a graduate student at Colorado State University working with Dr Cordain, the creator of the Paleo Diet. We are currently researching potential dietary triggers for autoimmune illnesses. As part of our research, we'd like to conduct confidential interviews with people who have an autoimmune illness and are on or have tried a Paleo-style or wheat-free diet for at least 6 months.

If you have been diagnosed with an autoimmune illness and are interested in being interviewed for this study, please contact me at [redacted]. The interview process should take no more than an hour and your answers will be kept confidential and anonymous. Our hope is that this research will shed more light on how diet can help combat autoimmune diseases. Your participation will be a great help.

Thank you,
Trevor Connor
Colorado State University

First Reply to Subjects Who Volunteer for the Study:
(please note that a sentence or two personal message may be included with the email)

Dear ,

Thank you for getting in touch with us and for being willing to participate in the study. It's appreciated. Right now we're in the process of identifying all of our volunteers. It should take us another 3 to 4 weeks to finalize our group of participants and then we'll be ready to begin the questionnaire process. I hope you don't mind if we ask you to wait while we go through this selection process.

I'll be in touch as soon as things are ready to go. Until then, don't hesitate to contact me if you have any questions. I'll look forward to talking with you further.

Thanks again for volunteering!

Trevor Connor
Department of Health and Exercise Science
Colorado State University