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Publisher's Letter

Change is in the air!

As I sit and reflect on what we have experienced for the past two years I realize how eager I am for a fresh start while embracing a special gift from Mother Nature; Spring! I recently had the pleasure of attending EXPO west hosted by New Hope in California. Fifty-five thousand attendees with no social distancing and no masks! In an environment I have spent my career thriving, I found myself feeling vulnerable and exposed. It hit me like a ton of bricks how entrenched the rules of pandemic life are set within me. I simply wasn't ready! Then I reflect on the very recent lifting of mask mandates in Ontario, and I watch fellow Torontonian's struggle as I did. Many choosing to keep their masks, one man describing a sensation of feeling naked without it, yet also those who feel comfortable removing them almost questioning why some people still wear them? Clearly, every individual is going to deal with post-pandemic life in their own way. It is going to be a highly individualized journey for every single person. It is to be expected, and respected. Each one of us has to recover at our own pace.

Then I think about the changes still to come. How will offices function? What will the virtual/in-person balance look like? How will supply chain issues impact patients and scope of practice? What will travel look like? People that know me know patience is not a gift I possess, yet the past two years has shown me I don't really have a choice!

With spring comes garden season. Our Editor-in-Chief, Dr Philip Rouchotas has a serious green thumb and has taught me a lot about how to create a productive garden. Looking forward to diving in and getting dirty!

On a final note I have the privilege of being involved in a process currently unfolding with Health Canada regarding changes to labelling of Natural Health Products. I look forward to keeping you informed!

Sanj
Sanjiv Jagota
Canadian Journal of Naturopathic Medicine
Publisher
publisher@cjnm.ca

Editor's Letter

Spring!

Ahhhhh… It's like a decompression… The first nice day in March hits, and it's as though the entire city (maybe country) just exhales in unison. For us in the Greater Toronto Area it was March 17. Sunny and 18 degrees!

Excited for lifting of pandemic restrictions, and hopeful their lifting endures. Got to take my kids skating in an indoor rink for the first time in two years! The past two years has made me take a step back a few times and realize that everyone deals with this crisis in their own way. The recent lifting of mask mandates in Ontario was yet another instance of needing to appreciate unique ways people are coping with this pandemic. Individuals choosing to wear masks are fearing bullying and ridicule from those whom have removed them. I have faith that people will be respectful enough to realize some people will simply need more time than others.

Very eager to get the garden going. Still far too early (one year I tried to start digging in early April and there were huge chunks of ice about an inch down). None-the-less, my kids are already putting in orders of what I am supposed to grow! So far I am obligated to deliver watermelons and potatoes beyond the typical basics. The list is sure to grow.

Our first issue of 2022 has Dr Hilborn delivering an excellent review of powerful links between insulin resistance and PCOS. Dr Nelson eloquently takes us back in time to string together the history leading to tremendous misinformation in the realm of acid-alkaline balance, then goes on to provide a thorough overview of modern perspectives of an important and often overlooked topic. Dr Mudry brings the concept of environmental stewardship to light in the context of botanical medicine while showcasing berberine as both a wonderfully effective and sustainable medicine. Dr Fritz delivers a comprehensive review of clinical applications of hops, a medicine that has received considerable research attention in the past 15 years. And CJNM is again thrilled to deliver a world class human intervention trial examining the ability of combinations of DHA, high-oleic canola oil, and barley beta glucan to impact cardiovascular disease risk factors.

Philip
Philip Rouchotas, MSc, ND
Canadian Journal of Naturopathic Medicine
Editor-in-Chief
editorinchief@cjnm.ca

Referees

CJNM would like to thank the following individuals for their efforts in serving as Referees, undertaking the process of Peer Review for this issue of the Journal.

Berchman Wong, ND
Adjust Your Health
Calgary, Alberta
berchman.nd@gmail.com

Chris Knee, ND
Infinity Chiropractic and Wellness Centre
Ottawa, Ontario
knee.christopher@gmail.com

Heidi Fritz, MA, ND
Bolton Naturopathic Clinic
Bolton, Ontario
info@boltonnaturopathic.ca

Jordan Morton, ND
Backfit
Victoria, British Columbia
drmorton@backfit.ca

Judith Ancheta, ND
Fertility Care Toronto
Toronto, Ontario
naturopathiccare@drjudynd.com

Lindsay Kellington, BHSc Kin, ND
Active Back to Health Centre
Calgary, Alberta
info@activebacktohealth.com

MJ Atkins, ND
Synergy
Victoria, British Columbia
dratkinsnd@gmail.com

Neil McKinney, ND (non-practicing)
Victoria, British Columbia
drneilmckinney8@gmail.com

Rachael O'Connell, ND
Medipro Holistic health
Beaverton, Oregon
Dr.OConnell@mediproholistichealth.com

Trent Brereton, ND
Kootenay Health Centre
Cranbrook, British Columbia
trentbrereton@me.com

Canadian Journal of Naturopathic Medicine

Dietary Acid Load: From Kimball C Atwood, the Father of Functional Food, to Contemporary Science

David H Nelson, ND (inactive)

David H Nelson, ND (inactive)
Woodstock, Ontario
davidhplanet@gmail.com

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Dietary Acid Load: From Kimball C Atwood, the Father of Functional Food, to Contemporary Science

Abstract

Much has been written about the “alkaline diet” in the lay press. Proponents suggest it is a cure-all for endless lists of human ailments. Critics suggest that since acid-base balance is so tightly regulated, the acidity or alkalinity of diet is, with only few exceptions, of little relevance to human health. This review explores early origins of the marketing of the acid-alkaline diet and reflect its rudimentary science off a more sophisticated, contemporary understanding of dietary acid load. As outlined here, the North American alkaline diet phenomenon was manufactured by grapefruit mogul and food faddist, Kimball C Atwood. The marketing techniques initiated by Atwood—using the trapping of science to promote a healthy idea to the masses—were highly lucrative and imitated by many other companies. However, by using the ornamentation of science, Atwood set in motion a century of confusion regarding the legitimacy of the acid-alkaline diet and the importance of dietary acid load in the prevention or management of chronic illnesses. Understanding this history allows naturopathic doctors to appreciate the reasons why confusing and contradictory media headlines related to acid-alkaline diets endure. The reasonable notion that dietary acid load and low-grade acidosis matters to various aspects of health—that it is neither a con nor a cure—is backed up by volumes of emerging science.

Introduction

Life on Earth has evolved under the influence of the potential of hydrogen (pH) within natural environments. For example, flourishing marine ecosystems have enjoyed a relatively stable ocean pH for millennia; however, rapid changes in the industrial age have already produced anthropogenic shifts in the pH of ocean water, threatening life within the marine ecosystems (Linares et al 2015, Pelejero et al 2010). In agricultural ecosystems, plant growth, and the presence of soil microbes that have a beneficial influence on plant growth, is determined by soil pH; the abundance and diversity of bacteria are increased along a pH range from acidic to slightly alkaline, and soil acidification, a threat to crop growth, is offset by various techniques in agriculture (Rousk et al 2010). Humans, of course, are no exception to the rule of hydrogen ion regulation; the pH of bodily systems is tightly regulated. For example, deviations from a very narrow 7.36-7.44 blood pH are considered an 'emia' (acidemia/alkalemia) and can present a threat to survival (Quade et al 2021).

The idea that specific dietary patterns and foods can put pressure on the tightly regulated pH within the blood and extracellular spaces has been contentious. In the first part of the 20th century, physicians placed a good deal of clinical emphasis on the pH of urine as a surrogate marker for various ailments; while the dangers of acidosis and alkalosis were well known, a tendency toward a more acidic urinary pH was much more frequently attached to chronic disease states. The general public responded to the emotional meaning of the word acid (from Latin acerbus (bitter); acidus (sour); acer (sharp)) and its perceived 'corrosiveness' to health; the notion of acid-base 'balance' was more likely to provided inner meaning and security to the lay consumer of information (Manz 2001).

The paper reviews the early origins of the marketing of the acid-alkaline diet and reflect its rudimentary science off a more sophisticated, contemporary understanding of dietary acid load. As outlined here, the North American alkaline diet phenomenon was initiated by grapefruit mogul and food faddist, Kimball C Atwood. Understanding this history allows the reader to appreciate the reasons why media headlines such as “The Alkaline Diet – Con or Cure?” (Walker 2011) endure. A century after Atwood invented the alkaline cure, Robert O Young, an uncredentialed “naturopathic practitioner”, wrote the international bestselling “*pH Miracle!*” (Young and Young 2002) which was translated into 18 languages. Young would eventually be convicted of practicing medicine without a license after he promoted cancer cures and set up alkaline intravenous treatments at $500USD per session (Figueroa 2017). The promotion of an alkaline diet as a “cure” or “miracle” is obviously not part of contemporary naturopathic training; however, the reasonable notion that dietary acid load and low-grade acidosis matters to various aspects of health—that it is neither a con or a cure—is backed up by emerging science.

Father of Functional Food and the Super Fruit

Early enthusiasm for the role of acid-alkaline influences and diet in health can be traced to Kimball C Atwood (1853-1936); Atwood was an influential entrepreneur who made heavy

investments in Florida's undeveloped west coast. In the early 1900s he transformed thousands of acres of land in Manatee County into the largest grapefruit grove in the world. At the time, widespread availability of grapefruit was limited. Atwood's challenge was how to sell those grapefruits and get a return on his massive investments. He decided upon an aggressive marketing campaign focused on the wide-ranging health benefits of grapefruit, and more specifically to promote it as "The New Cure" for disease. In his words: "Nature has responded to the world-wide cry for relief from a distressing malady by appealing to the palate in a most seductive way" (Atwood Grape Fruit 1910A).

Of course, patent medicines and botanical nostrums had long been sold for ailments from A to Z. However, Atwood broke from this tradition and chose a single dietary item and promoted its multidimensional healing properties. Seizing on the fledgling clinical interest in manipulating urinary pH to promote health, Atwood developed a three-pronged marketing campaign to sell his grapefruits. First, he touted physician support for the food. For example, "A prominent physician of New Haven prescribes [grapefruit] for all his patients, telling them be sure to get the ATWOOD" (Atwood Grape Fruit 1909); second, Atwood described the curative properties from diseases and disorders, and more generally for increased "energy", to "renew their youth" and inferences that it improves sleep ("taken at night on retiring it is better than drugs") (Scribner 1909); third, he provided at least one key mechanism of action by which the grapefruit could cure diseases and promote health - that is, by "rendering an unduly acid urine alkaline" (Atwood Grape Fruit 1910B).

The marketing campaign was directed at physician journals and the lay press. Indeed, Atwood paid for block advertisements in journals such as *American Medicine*. Perhaps delighted with his advertising support, the editors of *American Medicine* wrote a favorable review of grapefruit for health which Atwood subsequently recycled and quoted within his advertisements in the more prestigious *Journal of the American Medical Association* (JAMA) and in dozens of other journals and magazines; the JAMA ad states: "American Medicine says: 'Realizing the great value of grapefruit, the medical profession have long advocated its daily use, but it has only been in the past few years that the extraordinary curative virtues of this 'King of Fruits' have been appreciated. This dates from the introduction of the ATWOOD Grape Fruit, a kind that so far surpasses the ordinary grape fruit that no comparison can be made" (Atwood Grape Fruit 1912). Atwood also incorporated the testimonial of Edward E Keeler, a medical doctor and homeopath, who wrote glowingly of the grapefruit in his popular book "Here's How Health Happens" (Keeler 1912).

Remarkably, Atwood not only simultaneously promoted the grapefruit cure and alkalinity, he also informed the advertisement reader on the extent of his financial investments in 'science'. For example, the half-page advertisement in *New England* magazine (1910) reads: "Physicians [have found] that *only* ATWOOD Grape Fruit can be depended upon to impart [the] properties so beneficial to persons of acid natures, especially sufferers of rheumatism... The ATWOOD Grove,

at Manavista, Florida, where 250 acres are devoted to its scientific cultivation at an initial expenditure of over a quarter of a million dollars" (Epicures 1910).

In sum, Kimball C Atwood promoted grapefruit as a food for function and separated it from other fruits as the 'king'; he injected physician testimonials and positioned the curative fruit as the subject of medical and scientific investment. The ability of this super fruit to render the urine alkaline was a central part of the message. Atwood's return on marketing investments was tremendous; his methods of advertising in magazine and journals were so successful that media wrote headlines such as "How Atwood Sells His Fruit" (Bittinger 1910), "To Advertize Grapefruit" (Harris 1906), and "A Selling Plan for Grapefruit" (Editors 1910). The latter story reported that Atwood recuperated $3 dollars in profit for every $1 invested in his aggressive marketing. The next wave of Atwood's marketing campaign, exemplified by these words in an advertisement in the *American Journal of Clinical Medicine*, broadened and reinforced the medicinal properties of the fruit: "Last season we placed emphasis on the curative value of citric acid as found in the ATWOOD Grape Fruit. With the first suggestion of the use of this grapefruit in rheumatic and febrile conditions came a quick endorsement from physicians and the public...in a class by itself when used either as a luxury or medicinally...everything that science and experience was done" (Atwood Grape Fruit 1911).

The place of Kimball C Atwood in the annals of medical history and health marketing isn't merely a matter of intellectual curiosity; he greatly increased the public's interest in the "acid-alkaline" diet and the idea that there are 'functional' foods and super fruits; competitors who had been quietly selling oranges as a mere commodity were astonished at the public uptake of Atwood's advertising, commenting that he had driven unprecedented levels of demand over mere months compared to their efforts over years. His grapefruit cure campaign was lengthy - a decade after it began, Atwood was still placing block advertisements in magazines directed at women (Atwood Grape Fruit 1918). Put simply, Atwood was a catalyst in spreading the idea that 'acidity' matters. He pushed the notion onto physicians and in a circular system, pulled it from them. As a matter of historical interest, it is worth pointing out that Benedict Lust, widely held to be the founder of naturopathic medicine in North America, was a rival of Atwood; Lust had his own grapefruit groves two hours away, in Tangerine, Florida. Lust marketed his grapefruits as a cut above in quality (Lust 1916).

Atwood's highly effective marketing campaign predated the first thorough attempts to identify the acid-base forming elements in common foods by several years; Henry C Sherman, who would go on to become the president of the American Institute of Nutrition and the American Society of Biological Chemists, and colleagues provided the first comprehensive study of 47 different foods - and the biological effects of consumption - in 1912 (Sherman and Gettler 1912). In the years following Atwood's marketing campaign, scientists and medical doctors in North America and Europe provided case reports supporting the use of specific diets that were purported to be 'alkaline'. For example, it was reported that the incorporation of alkaline foods reduced cutaneous inflammatory lesions (Kroetz 1930, Umrath 1943). Researchers in

dermatology concluded that “In nearly all cases, active inflammatory processes cease and the eruption rapidly clears when the urine is rendered alkaline” (Ormsby and Mitchell 1920). By the late 1930s, an alkaline diet was recommended for inflammatory skin conditions such as eczema (Hellier 1938). However, there were mixed findings in the research (Ingram and Fowweather 1931, Whitfield 1934) and the validity and relevance of the acid-alkaline food lists was questioned (Bischoff et al 1934); milk company scientists seemed especially sensitive, claiming that the acid-alkaline diet was a major part of food fads promoted by “quacks and ignoramuses and religiously followed by a host of deluded persons” (Tobey 1936). By the 1960s, outside of metabolic acidosis or kidney disease, the acid-alkaline diet was dismissed as being of little relevance to clinical nutrition (Brady 1959, Ewalt 1959).

Dietary Acid Load

The acid-alkaline diet concept emerged again in the 1970s. In 1969, two physician/scientists writing in the *Lancet* proposed that osteoporosis may, to some degree, be related to the dominance of acidic foods (or the absence of alkaline fruits and vegetables) in the western diet. They proposed that in the context of a continuous acidic, western diet, the tight regulation of blood pH necessitates miniscule amounts of alkaline salts to be removed from bone as a buffering source. They suggested that over time this could add up to meaningful losses—as much as 15% of inorganic bone mass in an average individual over a decade (Wachman and Bernstein 1968). Until the end of first decade of the 21st century, there was little evidence to support the theory. However, as discussed below, new evidence has substantiated the hypothesis.

One of the potential factors behind the inconsistency of findings related to early acid-alkaline diet interventions, and the topic in general, was related to the inaccuracy of lists of supposed acid and alkaline foods and beverages. Early on, the determination of the acid or base potential of a food or beverage in the human body was based on subjecting the food to combustion and determining if the ash was acid or alkaline (Dwyer et al 1985). However, the laboratory test known as the potential renal acid load (PRAL), developed in 1995, is now recognized as a reliable indicator of the acid or alkaline potential of a food or beverage in the human body. Foods and beverages rich in proteins and sodium are said to have a high, or more positive PRAL. That is, they are considered to be acidic in the human body. On the other hand, foods and beverages that are rich in potassium and alkaline minerals with the potential of forming bicarbonates are said to have a lower, or more negative, PRAL score (Remer and Manz 1995). Citrus fruit as promoted by Atwood is definitely in the negative range; the consistent theme is that fruits and vegetables are alkaline (negative PRAL), meats, hard cheeses and grains are acidic (positive PRAL), and milk and yogurt are mildly acidic. PRAL scores are a reliable marker of dietary acid load and shifts in urinary pH reflect PRAL-associated dietary patterns (Remer et al 2003).

While the acidic diet osteoporosis theory has been historically contentious, recent studies clearly demonstrate that an alkaline diet (plant-based, low-PRAL) and dietary alkaline supplements are capable of increasing urinary pH, blood pH, and/or blood bicarbonate concentrations (Cosgrove

and Johnston 2017, Hottenrott et al 2020, Limmer et al 2020). Well-designed studies published in the last five years provide evidence that high PRAL and net endogenous acid production (NEAP) are negatively associated with bone mineral density, and positively associated with frailty and fracture risk (García-Gavilán et al 2021, Hayhoe et al 2020, Kataya et al 2018). The findings are bolstered by studies that have linked fruit intake and serum bicarbonate levels to bone mineral density in older adults (Liu et al 2015, Tabatabai et al 2015), and alkaline salt (potassium) interventions or potassium-rich dietary patterns that show a favourable influence on bone health (Cao et al 2021, Granchi et al 2018, Ha et al 2020, Moseley et al 2013).

While the connections between dietary acid load and bone health continue to strengthen, there has been a robust growth in research linking dietary acid load to multiple chronic non-communicable diseases. In the last five years, dietary acid load has been associated with type 2 diabetes (Akter et al 2017, Kiefte-de Jong et al 2017), insulin resistance (Caferoglu et al 2021, Gæde et al 2018, Lee and Shin 2020, Moghadam et al 2016), obesity (Abbasalizad Farhangi et al 2019), cardiovascular disease and associated lipid risk factors (Arisawa et al 2020, Han et al 2016, Jafari et al 2021, Mazidi et al 2018), hypertension (Banerjee et al 2021, Krupp et al 2018, Murakami et al 2017), hyperuricemia (Esche et al 2018, Shin and Lee 2021), kidney dysfunction (Mirmiran et al 2016, Rebholz et al 2015, So et al 2016), breast cancer (and prognosis) (Park et al 2019, Wu et al 2020A), pancreatic cancer (Shi et al 2021), lung cancer (Ronco et al 2021), colorectal cancer (Jafari Nasab et al 2021), migraine headaches (Mousavi et al 2021), and mortality (Akter et al 2017). Discussed in further detail below will be the remarkable new studies linking dietary acid load to mental disorders. With so many non-communicable diseases linked to dietary acid load, it should be expected that there should be one or several common mechanisms of action. As discussed below, the idea that, in the context of our evolutionary past, the acidic westernized diet is a metabolic stressor, is one unifying concept.

Acidic Diet as a Stressor

The theory that a consistently acid westernized diet places a burden on the skeletal system (a reservoir of alkaline minerals) has provided a relatively sound mechanism of action for links between diet and osteoporosis; however, until recently, mechanistic links between dietary acid load and many diverse medical conditions have been elusive. In a 2003 study by Swiss researchers, a specific physiological change induced by a high-acid load, fast-food type diet was discovered. Consumption of an acidic, western-type diet for nine days significantly elevated the stress hormone cortisol. When researchers neutralized the diet with bicarbonate supplements, even while subjects maintained the high-acid-load diet, the cortisol levels were reduced significantly versus controls maintaining the western diet (Maurer et al 2003). Similar clinical studies have since demonstrated the ability of oral alkaline supplements to neutralize elevations in glucocorticoid activity (Buehlmeier et al 2016, Conen et al 2016). In children, increased fruit and vegetable intake has been associated with lower urinary glucocorticoids (Esche et al 2015, Esche et al 2016).

Essentially, an acidic, westernized diet, largely devoid of potassium, bicarbonates and other alkaline-rich fruits and vegetables, is a metabolic stressor. In the context of our evolutionary past, such a diet is relatively novel and may present a 'mismatch'. Since subclinical hypercortisolism with chronic, mild cortisol excess is frequently related to hypertension, and is also characteristic of obesity, cardiovascular disease, lipid disorders, type 2 diabetes, lower skeletal muscle mass, various mental disorders, and osteoporosis, the implications to healthcare are significant (Di Dalmazi et al 2015, Herane-Vives et al 2018, Kim et al 2018, Min 2016, Ortiz et al 2019). In line with this research is the recent finding that higher dietary acid load is associated with C-reactive protein (CRP)— compared to women with the lowest quartile of dietary acid load, women with the highest quartile showed 30–33% higher levels of CRP (Wu et al 2019). The relationship between dietary acid load, glucocorticoids, and inflammation, now replicated in several studies, represents at least one important unifying mechanism whereby the absence of alkaline-rich plant foods can be related to multiple chronic non-communicable diseases.

Dietary acid load has recently been linked to depression, anxiety, and other mental disorders (Daneshzad et al 2020, Milajerdi et al 2020, Mozaffari et al 2020, Wu et al 2020B). For example, depressed women with a dietary acid load higher than median reported 2.75 times the risk of reduced physical function and 3.10 times the risk of poor physical health compared to non-depressed women with a dietary acid load lower than median (Tessou et al 2021). Children with high PRAL dietary patterns have been found to have more overall emotional problems, and hyperactivity in particular (Bühlmeier et al 2018). Even a short-term (two week) high-potassium/low-sodium dietary intervention has been shown to improve mood compared to control diets (Torres et al 2008).

Additional mechanistic pathways involve alteration of the gut microbiome. For example, alkaline water, compared to tap water, has been shown to increase the growth of *Bifidobacterium* (Tanaka et al 2021). Emerging clinical research demonstrates value of alkaline water in irritable bowel syndrome (Shin et al 2018); animal research suggests that alterations in microbial tax may be at least one mechanism by which the alkaline water positively influences the intestinal ecosystem (Higashimura et al 2018).

Gone are the days when dietary acid load and acid-base balance can be dismissed as irrelevant. In 2021, the results from the Golestan Cohort Study with over 50,000 participants showed that acid and alkaline balance are important to cardiovascular and all-cause mortality. The researchers concluded that "it may be important to consider a balanced acid-base diet as a protective strategy to prevent pre-mature death, especially from cardiovascular disease" (Hejazi et al 2021). Recent randomized controlled trials demonstrate that plant-based diets significantly reduce dietary acid load (Kahleova et al 2021, Müller et al 2021).

Cancer Controversy

Among the lay public, the concept of dietary acid load is most often discussed in relation to cancer prevention and/or treatment (Walker 2011). In recent years, a spate of trade paperbacks have been published with titles promising the prevention, cure and/or management of cancer by an alkaline diet (Doris 2020, James 2020, Josh 2021, Philip 2021). Despite the epidemiological links between dietary acid load and cancer, there is at present no research that shows that specific dietary interventions are helpful in oncology by virtue of an alkaline potential. At the same time, the widely touted skeptical claim that the systemic microenvironment surrounding tumors cannot be altered by oral alkaline interventions, has itself been debunked. In animal models, researchers have proven that oral bicarbonate, without changing blood pH, can increase the extracellular pH of tumors. The reduced acidity at the local level subsequently decreased the *in vivo* number and size of tumor metastases, as well as survival (Robey et al 2009). Recently, low levels of serum bicarbonate have been associated with higher levels of systemic inflammation and lower short-term survivability in post-operative adults with colorectal cancer (Chan et al 2020). The use of bicarbonate and other alkaline-based interventions to treat cancer is currently an area of intense scrutiny by oncology researchers (Ando et al 2021, Wang et al 2021).

This exciting work should not be confounded, as it often is by the lay public and those promoting profit-based books and over-the-counter products, with an alkaline diet cure. In 2021, the website Plant Based News reported the case of a cancer cure through an alkaline diet intervention, but that story was subsequently retracted by the outlet (Baker 2021).The story and its retraction encapsulate an ongoing controversy that is nearly a century old. For now, the benefits of promoting a diverse, colourful, fibrous diet dominated by polyphenol-rich fruits and vegetables to the wellbeing of all individuals, including those with a history of cancer, with current cancer, or in remission, are obvious and many.

Conclusion

The idea of the industrial diet as harmfully acidic, and a functional superfruit to provide a healthy alkaline offset, belongs to citrus mogul Kimball C Atwood. The marketing techniques initiated by Atwood—using the trapping of science to promote a healthy idea to the masses—were highly lucrative and imitated by many other companies. However, by using the ornamentation of science, Atwood placed a decorative horse before the grapefruit cart, and set in motion a century of confusion regarding the legitimacy of the acid-alkaline diet and the importance of dietary acid load in the prevention or management of chronic illnesses. Despite volumes of recent scientific evidence on the importance of dietary acid load, and the mechanisms linking it to chronic disease (and dis-ease), the concept is still dismissed in some quarters as a fad.

Knowledge of the importance of acid-base balance and the consequences of metabolic acidosis or metabolic alkalosis is an important part of medical training. In 2021, an editorial in *BMJ Open Heart* stated that low-grade acidosis, the version induced by the westernized diet, is a "21st

century public health crisis" requiring greater emphasis in medical schools (DiNicolantonio and O'Keefe 2021). Naturopathic doctors are well-versed in the topic, and accountability can be found in required testing in both basic science and clinical exams. At the same time, naturopathic doctors have been keen observers and clinical specialists in the rational center of a long continuum—between the poles of miracle cures at the one end, and outright dismissal of the acid-base balance by dairy board propagandists at the other. As the evidence mounts on the relationships between dietary acid load, chronic low-grade acidosis/hypercortisolism, and chronic non-communicable diseases, naturopathic doctors are ideally positioned to provide clinical guidance.

Beyond the clinic, it is important for our profession to separate ourselves from the pseudoscience associated with "alkaline diets" touted to cure long lists of ailments and "melt the pounds away". The 2017 sentencing agreement in the State of California vs. Robert O Young, the international celebrity of the alkaline diet, was that he make a public admission "declaring that he is not a microbiologist, hematologist, medical or naturopathic doctor or trained scientist" (Figueroa 2017). As licensed and trained naturopathic doctors, it is vital that we understand the borderland between Kimball C Atwood's superfood marketing, and the emerging science that can promote the health of our patients and communities.

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Insulin Resistance in Polycystic Ovary Syndrome (PCOS): Evaluation and Important Associations

Andrea Hilborn, ND

Andrea Hilborn, ND
Kingston, Ontario
andrea@drandreahilbornnd.com

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Abstract

PCOS is common, and as understanding evolves to recognize many diverse presentations, prevalence of PCOS continues to increase. There are clear associations of tremendous interest to the clinician between PCOS and an array of common patient complaints, and insulin resistance seems to play a key role in most such presentations. The gold-standard tool for assessing insulin sensitivity, the euglycemic-hyperinsulinemic clamp technique is reserved for research purposes and is not broadly available as a diagnostic test. This review will showcase associations between PCOS, insulin resistance, and several important common patient concerns. The review will also attempt to determine if an adequate alternative to the euglycemic-hyperinsulinemic clamp technique exists for accurate determination of insulin resistance in outpatient practice.

Introduction

The prevalence of polycystic ovarian syndrome (PCOS) is high. Insulin resistance is present in PCOS at a higher rate than in the general population, therefore, it benefits the clinician to understand insulin resistance in the context of PCOS. This article will review the prevalence of insulin resistance, the clinical outcomes associated with it, and the measurement of insulin resistance specifically within patients who meet the diagnostic criteria for PCOS. Given the ability of insulin resistance to predict many negative outcomes or comorbidities in patients with PCOS, in-office tools to accurately determine insulin resistance would be very valuable.

Prevalence and Severity of Insulin Resistance in PCOS

Insulin resistance is present in most cases of PCOS. A 2016 meta-analysis, which looked exclusively at results of euglycemic-hyperinsulinemic clamp studies including 1224 cases and 741 controls, found that insulin sensitivity was 27% lower in women with PCOS. They did not report on the rate of insulin resistance among PCOS cases, nor distinguish the severity of insulin resistance at different BMIs. Elevated BMI had a greater effect on insulin sensitivity in cases than in controls. BMI had a greater impact on insulin sensitivity than did PCOS (Cassar et al 2016).

Another meta-analysis conducted in 2017 found that 74.9% of those meeting Rotterdam criteria for PCOS were insulin resistant as determined by euglycemic-hyperinsulinemic clamp. The percentage of insulin resistant subjects was 59.3% in normal-weight, 77.5% in overweight, and 93.9% in obese patients (Rezvanian et al 2009).

A study published after these meta-analyses, using euglycemic-hyperinsulinemic clamp, which had 448 cases and 40 controls, found a rate of insulin resistance of 56.3% in women with PCOS. Insulin resistance was present in 18.8% of lean women with PCOS. This study included Chinese women only (Li et al 2019).

A 2019 meta-analysis found that in nonobese persons with PCOS, the odds ratio of insulin resistance was 5.70 (1.46-22.32). However, the study did not make clear how insulin resistance was measured. Only three studies met their criteria for analysis of insulin resistance (Zhu et al 2019).

The quality of the information about prevalence and severity of insulin resistance in PCOS is low relative to the worldwide incidence of the syndrome. From the available studies, we can conclude that most people with PCOS are insulin resistant.

Androgen Levels and Insulin Sensitivity

A small amount of evidence links severity of insulin resistance with severity of hyperandrogenism.

In a small study of 27 people with PCOS, testosterone levels were associated with the Matsuda index of insulin sensitivity, independent of BMI (Luotola et al 2018).

A larger study of 378 PCOS subjects found that free androgen index (FAI) was negatively related to HOMA-IR r = -0.413 p <0.001. Across tertiles of FAI, insulin sensitivity decreased (Zhang et al 2018).

Similarly, in a study of 1000 PCOS cases, FAI was significantly correlated with fasting insulin (FIN) and HOMA-IR (r = 0.240, P < 0.001 and r = 0.191, P < 0.001, respectively). Free testosterone was significantly correlated with FIN after adjusting for the influence of age (Zhang et al 2019).

In a study of 448 cases and 40 controls, insulin resistance as determined by euglycemic-hyperinsulinemic clamp was associated with testosterone levels, but insulin resistance as determined by HOMA-IR was not (Li et al 2019).

To summarize; when PCOS gets worse, IR gets worse.

This small body of evidence does not explain the nature of the relationship between insulin resistance and hyperandrogenism, which remains an important nut to crack in understanding PCOS.

Insulin Resistance in PCOS and Outcomes of Interest

Some of the body of evidence exploring PCOS has examined the relationship between outcomes of interest to patients and insulin resistance. These studies all use surrogate markers of insulin resistance, as opposed to euglycemic-hyperinsulinemic clamp studies, which means they are likely underestimating prevalence and severity of insulin resistance, as will be discussed below. Studies looking at incidence of metabolic syndrome in PCOS have not been included here, as they fail to report specifically on insulin sensitivity of their subjects.

Fertility

In 1000 PCOS cases, the odds ratio of live birth was 1.81 in the group with HOMA-IR <2.69 compared to HOMA-IR >/= 2.69 (1.26, 2.55, p= 0.001). Live birth is the ultimate outcome for fertility. However, this study also found statistically significant correlations between fasting insulin and ovulation per ovulation induction cycle, conception, and pregnancy. No associations were found between fasting insulin or HOMA-IR and miscarriage (Zhang et al 2019).

Depression

PCOS patients are at a three to four-times greater risk of depression and five to six-times greater risk of anxiety than those without PCOS (Cooney et al 2017, Greenwood et al 2018). In one study, there was a significant association between HOMA-IR and depression, but no association between HOMA-IR and anxiety. In a study of 738 PCOS cases, HOMA-IR >2.2 was associated with a 2.3-fold increased risk of depression (Greenwood et al 2018).

Sleep Apnea

A systematic review including six studies found that subjects with PCOS and obstructive sleep apnea (OSA) had higher HOMA-IR than those with PCOS but without OSA (Kahal et al 2018).

Hirsutism

A controlled trial of laser hair removal compared to laser hair removal plus metformin 500mg tid found that in the metformin group, HOMA-IR decreased from 2.83 to 1.35, and that the metformin group had superior response to laser hair removal (Rezvanian et al 2009).

Bone Mineral Density

Bone mineral density appears to be an outlier; worse insulin sensitivity is associated with higher bone mineral density. However, in the single study examining insulin sensitivity in PCOS, the controls had a very high mean HOMA-IR of 3.6 (Pereira-Eshraghi et al 2019).

Measuring Insulin Resistance in Practice

The high prevalence of insulin resistance in PCOS, as well as its association with hyperandrogenism and with outcomes of clinical significance, demonstrates the desirability of being able to measure insulin resistance in clinical practice. To this end, we will review the reliability of surrogate markers of insulin resistance in the context of PCOS. We will additionally attempt to find any useful cutoffs for those markers.

Seeing average HOMA-IR scores in PCOS compared to healthy controls can help orient us. A meta-analysis compared HOMA-IR in 3037 subjects divided into four groups: obese/PCOS, non-obese/PCOS, obese/non-PCOS and non-obese/non PCOS. The pooled mean of HOMA IR in each group was 4.38 (3.84, 4.92), 2.68 (2.16, 3.20), 2.44 (2.06, 2.82) and 1.34 (1.06, 1.63) respectively. The difference between non-obese/PCOS and obese/non-PCOS was not statistically significant (Behboudi-Gandevani et al 2016).

HOMA-IR is relatively simple for a clinician to calculate. Fasting insulin (mU/L) is multiplied by fasting glucose (mg/dL). A value between 0.5 and 1.4 denotes good insulin sensitivity. Higher scores denote increasingly poor insulin sensitivity (The Blood Code 2022). This simple calculation, which can be easily performed in-office, provides a decent marker of insulin sensitivity, yet unfortunately, as reviewed below, significantly underestimates insulin resistance relative to the euglycemic-hyperinsulinemic clamp technique.

A 2019 study of 448 cases and 40 controls comparing the euglycemic-hyperinsulinemic clamp to HOMA-IR found the Kappa value to be 0.069 for lean PCOS subjects and 0.139 for obese PCOS subjects, where a Kappa value of zero indicates no relationship. The prevalence of IR estimated by HOMA-IR was lower than that determined by clamp technique. By clamp technique 34.5% of lean PCOS subjects and 80.2% of obese PCOS subjects were insulin resistant. Using HOMA-IR, 15.6% of lean PCOS subjects and 41.6% of obese PCOS subjects were insulin resistant (Li et al 2019).

Another 2019 study with 537 subjects found a much more promising correlation coefficient of r= -0.6828 when comparing the euglycemic-hyperinsulinemic clamp and HOMA-IR results (Long et al 2019).

The 2017 Tosi et al study that found a high rate of 74.9% insulin resistance in PCOS subjects also looked at the euglycemic-hyperinsulinemic clamp compared to surrogate indices. The percentage of PCOS subjects with IR was lower as determined by HOMA-IR than by clamp, at 41.1%. The correlation coefficient of HOMA-IR and the euglycemic-hyperinsulinemic clamp was r= 0.622 p<0.001. Going a step further, the authors reported positive and negative predictive values, concluding that for all surrogate markers, specificity for detecting insulin resistance was fair but sensitivity was low (Tosi et al 2017).

There are a couple of smaller studies as well, both specifically in adolescents.

A 2018 study of 28 adolescents with PCOS found the following correlation coefficients with comparison to the euglycemic-hyperinsulinemic clamp: HOMA-IR r= -0.78, p<0.001, e-IS r= 0.70, p<0.001, Matsuda r= 0.533, p<0.001 (Cree-Green et al 2018).

A 2020 study comparing the euglycemic-hyperinsulinemic clamp to various surrogate indices in 26 adolescents with PCOS found the following correlation coefficients: oral minimal model estimation of insulin sensitivity r=0.64, p<0.0001, oral minimal model estimation of dynamic insulin sensitivity r=0.73 p <0.0001, Matsuda index r=0.59 p<0.003 (Carreau et al 2020).

Beauty is in the eye of the beholder; a desirable correlation coefficient in this case lies with the judgment of the clinician. There is considerable variability. The worst result was in lean PCOS subjects. It is probably safe to say that surrogate markers should not be used to rule out insulin resistance, since all surrogate markers appear to underestimate the rate of insulin resistance.

Other Possibilities

Fortunately, the search for a solution for the clinician looking to understand the level of insulin sensitivity in a particular patient continues.

Inferring insulin resistance based on sex hormone binding globulin levels may prove to be an acceptable workaround. In a study of 100 PCOS patients and 61 controls, in the non-overweight group, a SHBG level of < 38.4 nmol/L predicted HOMA-IR >2.29 with 92.86% sensitivity (95% CI: 82.7–98.0%) and 74.39% specificity (95% CI: 63.6–83.4%) (Chen et al 2021).

Many diverse molecules are being investigated for their utility in identifying insulin resistance, although none are sufficiently well-established for clinical use yet (Polak et al 2017).

Conclusion

Insulin resistance is present in most patients with PCOS, and strongly predicts the presence and severity of common comorbidities associated with PCOS. It would be beneficial if the clinician could accurately assess insulin resistance in individual patients in order to treat only those who

require it, and to gauge improved risk of various undesirable outcomes. HOMA-IR, although widely used in studies looking at outcomes of importance to PCOS patients, correlates moderately with insulin resistance determined by the euglycemic-hyperinsulinemic clamp, but does not correlate perfectly. It certainly underestimates insulin resistance, and may underestimate it drastically. Other surrogate markers perform similarly and are less frequently used in studies of clinical outcomes. Researchers are working diligently to provide a better solution.

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Immunomodulatory and Anti-Inflammatory Actions of Berberine-Containing Herbs

Katelyn Mudry, ND, MSc

Katelyn Mudry, ND, MSc
Dr Katelyn Mudry Naturopathic Medicine
Cranbrook, BC
info@drkmudry.com

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Immunomodulatory and Anti-Inflammatory Actions of Berberine-Containing Herbs

Abstract

Sustainability of commonly employed medicinal plants is becoming a global concern, heightened by shortages imposed during the current pandemic. Berberine-containing plants are found broadly in nature, present across several families of plants and potentially cultivated almost anywhere in the world. Research in berberine has exploded in recent years, leaving a well-developed body of good quality evidence demonstrating a wide array of important clinical outcomes across a broad spectrum of applications. This review will showcase important roles of berberine from both modern research and traditional herbal systems of medicine. The review will also raise important concepts relating to herbal medicine sustainability and demonstrate that berberine ranks very high when considering environmental stewardship while seeking safe and effective herbal medicines.

Introduction

With the prevalence of chronic disease and inflammation on the rise, we have seen a boom in natural anti-inflammatory treatments. The most renowned anti-inflammatory treatment is *Curcuma longa* (turmeric), a bright yellow rhizome that is native to India and southeast Asia. Modern research has highlighted curcumin, a constituent in turmeric, as an anti-inflammatory agent and it has been well-studied in multiple inflammatory conditions such as autoimmune diseases and cancer (White et al 2019). *Curcuma* is in high demand worldwide and is sourced commercially almost exclusively from its native areas (Schippmann et al 2002). Recently (2020-current) we have seen wide scale disruptions in supply chains and the ability to import products such as *Curcuma* ethically and consistently. Concerns around environmental impacts and sustainability of mono cultivation, as well as quality of large-scale commercially grown crops and a growing interest in bio-regional herbalism have recently come to prominence. This creates a need to identify more anti-inflammatory herbs that are local to practitioners' areas (Schippmann et al 2002). Turmeric's traditional use is for digestive conditions such as gallstones, jaundice, colic and flatulence, and blood sugar support. It has also traditionally been used as an anti-inflammatory for arthritis, menstrual pains, and topically for skin issues such as eczema (Khalsa and Tierra 2008, Tierra 1988). In looking for North American herbs with a similar traditional profile to turmeric we find *Mahonia aquifolium* (Oregon Grape root) and *Hydrastis canadensis* (Goldenseal).

Mahonia and *Hydrastis*, native to western and eastern North America respectively, also have bright yellow rhizomes and have very similar traditional uses to *Curcuma* (see below). The main identified constituent in these two plants is berberine. Berberine is an isoquinoline alkaloid found in many different herbs across multiple continents and plant families. Berberine-containing herbs are commonly known as digestive tonics, bitter stimulants, anti-microbials and immune stimulants (Yarnell 2004). Some of the more commonly known herbs include: the *Berberidaceae* family - *Berberis* spp. (Barberry) found in Europe, the Middle East and India and *Mahonia* in North America; the *Annonancea* family - *Annickia chlorantha* (African whitewood) in west Africa; the *Papaveraceae* family - *Argemone mexicana* (Prickly Poppy) in Mexico and *Chelidonium majus* (Celandine poppy) from Europe and western Asia; the *Ranunculaceae* - *Coptis chinensis* (Chinese goldthread) from China and *Hydrastis* in North America; and the *Rutacea* - *Phellodendron* spp. (cork tree) in eastern Asia to Japan (Ehteshamfar et al 2020). This widespread cultivation range and diversity of berberine-containing herbs makes them a sustainable and locally sourced choice for herbal treatment worldwide.

Traditional Usage of Berberine-Containing Herbs

Most berberine-containing herbs are traditionally used for liver, gallbladder, and metabolic support, as well as a gastrointestinal and dermatological anti-microbial (Ehteshamfar et al 2020, Wood 2008, Wood 2009).

Multiple plants have specific anti-inflammatory and immune related traditional uses as well, such as:

European herbs *Chelidonium* for migraines, myalgias and abdominal cancer; and *Berberis* spp for chronic inflammation, throat and tonsil swelling/inflammation/infection, eczema and psoriasis and acute and chronic infection.

North American herbs *Hydrastis* for insulin resistance, colitis, cystitis, and multiple inflammatory skin conditions and *Mahonia* for allergies, Crohn's disease, psoriasis, and both osteo and rheumatoid arthritis (Wood 2009).

The Chinese herb *Coptis* for acute and chronic inflammation and infection, gynecological, and urinary inflammation and abscess, boils, and burns (Bown 1995, Buhner 2012).

Indian barberry, *Berberis aristata* (Tree turmeric) for spleen inflammation, fever, bacterial infections, and gallbladder inflammation (Khalsa and Tierra 2008).

The current evidence base has broad support for berberine use in certain conditions. Berberine has been well studied for its metabolic effects and is commonly used in treatment of diabetes, hyperlipidemia, and other metabolic conditions (Ye et al 2021). Additional research has been done on its effect on metabolic and inflammatory pathways in patients with polycystic ovarian syndrome (PCOS) (Kuang et al 2020). Its antimicrobial actions have been seen with a plethora of different microbes from *streptococcus spp* to *Treponema pallidum* (syphilis) to *Giardiasis* and other amoebas and parasites (Buhner 2012, Kane 2017, Wood 2009).

Inflammatory Outline

Chronic inflammation can arise through multiple pathways. The main causes of chronic inflammation include persistent pathogens, auto immune reactions, and foreign bodies (Beg et al 2011). Chronic inflammation can also be initiated by an unresolved episode of acute inflammation (Hall 2011). Acute inflammation is the response to cellular or vascular injury from trauma or invasion of pathogens. Acute inflammation includes many different cell types including endothelial cells, macrophages, and signaling and stimulating factors. Most notable of these factors are the pro-inflammatory cytokines Interluekin-1 (IL-1), tumor necrosis factor (TNF), and IL-6 (Chapel et al 2008). Chronic inflammation can also arise from persistent infections, hypersensitivity reactions, allergens, and exposure to toxic agents (Kumar et al 2015). The body's attempts to resolve or remove the insulting factor leads to continuous signaling of the inflammatory cascade and increased immune reaction intensity. This over-abundance of the immune response can then lead to significant tissue damage. Macrophages are key cells in chronic inflammation, releasing products such as reactive oxygen species (ROS) and transforming growth factor beta (TGF-β). Activated macrophages release TNF, IL-1, and other cytokines, drawing more cells to the affected area. T cells are stimulated by the macrophage response and the produced cytokines, in particular IL-12. Activated T cells release TNF, IL-17, and other cytokines to recruit macrophages and interferon gamma (IFN-γ) which activates them.

In many conditions, especially auto immune conditions, different subsets of T cells are known to be highly inflammatory and correlate to disease expression. These include T helper type 1 (Th1) and Th17 in particular with T regulatory cells (Tregs) helping to modulate inflammation (Ehteshamfar et al 2020, Shen et al 2020). While this author recognizes there are multiple other chemical and hormonal factors that can influence inflammatory states, they are outside the scope of this review. This review will focus on a selection of commonly studied and measured cytokines and immune cells mentioned above.

Metabolic Inflammation

As a metabolic therapeutic, berberine improved dyslipidemia and insulin resistance in multiple human trials (Lan et al 2015, Ye et al 2021). Much of berberine's effect on glucose uptake is through adenosine monophosphate-activated protein kinase (AMPK). AMPK has antioxidant effects which in turn decrease inflammation (Cicero and Baggioni 2016). IL-6, TNF-α and COX-2 additionally play a large role in diabetes and atherosclerotic pathology (Chang et al 2015, Rui et al 2021). Berberine has been shown to protect endothelial cells by lowering IL-6 and TNF-α and attenuating NF-κB signaling (Rui et al 2021). Berberine has also been shown to inhibit cycoloxygenase-2 (COX-2) activity and prostaglandin E2 (PGE2) activity (Liu et al 2015). Modulation of these inflammatory pathways is additionally enhanced by berberine's ability to influence monocyte mobility and macrophage activity (Rui et al 2021).

Polycystic Ovary Syndrome (PCOS)

Most berberine and PCOS studies focus on the metabolic markers of PCOS (Rondanelli et al 2020, Xie et al 2019). Berberine alone as a treatment can improve ovulation and menstrual patterns in PCOS patients (Li et al 2015B). A systemic review showed berberine to be superior to metformin treatment for metabolic markers as well as hormone modulation (Xie et al 2019). The pathophysiology of PCOS is far more complex though then just insulin resistance and metabolic changes (Zhang et al 2021). Inflammation in PCOS patients is considered to be chronic and low-grade (Kuang et al 2020). Chronic low-grade inflammation is a pattern seen in multiple forms of chronic disease including obesity, asthma, chronic fatigue, inflammatory bowel disease, and more (Zhong and Shi 2019). The PCOS inflammatory picture is characterized by high levels of leukocytes, IL-6, and increased activation of Th-17 cells (Rudnicka et al 2021, Yang et al 2021). A murine model of PCOS showed widespread anti-inflammatory actions with berberine treatment. Significant decreases in expression of multiple inflammatory markers including TLR4, NF-kB, TNF-α, IL-1, and IL-6 were seen (Shen et al 2021). Not all people with PCOS have global or central adiposity, though those who do have increased inflammatory production from adipose tissues as well (Wawrzkiewicz-Jalowiecka et al 2020). Changes in body composition and visceral fat have been seen which have an anti-inflammatory effect (Wawrzkiewicz-Jalowiecka et al 2020, Wei et al 2012). Treatment with berberine decreased hs-CRP in a small PCOS clinical trial (Cicero et al 2014).

Autoimmune Actions

Berberine has displayed the ability to modulate Th17/Treg responses in multiple autoimmune conditions including rheumatoid arthritis (RA) and experimental colitis, type 1 diabetes, and myocarditis (Shen et al 2020). Berberine's effects on autoimmune conditions are directed through Th1/Th17 modulation and indirectly through regulation of Tregs, macrophages, and dendritic cells (Ehteshamfar et al 2020). Modulation of Th1 and Th17 responses has been linked to clinical progression and severity of auto immune disease. Decreases in Th1 and Th17 cells reduced levels of pro-inflammatory cytokines and thus decreased autoimmune responses (Ehteshamfar et al 2020). Reduction of these cells in experimental autoimmune myocarditis and colitis showed improvements in respective organ function and decreases in pathophysiology (Li et al 2015A, Liu et al 2016).

Murine models repeatedly show decreases in multiple cytokine pathways and direct decreases in Th1 and Th17 cells with berberine administration (Marinova et al 2000, Yue et al 2017). In rheumatoid arthritis models, berberine extract has demonstrated the ability to decrease the inflammatory cytokines IFN γ, IL-17, and IL-2, suggesting actions on both the humoral and cell mediated immune pathways (Hu et al 2011). Multiple additional immunomodulating actions of berberine in relation to RA have been found including apoptosis, inhibition of NF-κB and dendritic cells, and modulation of macrophages and Th17/Treg cell responses (Shen et al 2020). Berberine has also been shown to suppress TNF-α, IL-6 and MCP-1, decrease levels of PGE2, down regulation of cyclooxygenase-2 (cox-2) and inhibit mitogen-activated protein kinase signaling (Ehteshamfar et al 2020).

For a detailed table of berberine actions on specific tissues see Ehteshamfar et al 2020.

Cancer

A 2022 review by He et al gives a very thorough review of the multiple cancer pathways *Coptis* and berberine extracts work on across multiple different cancer types (He et al 2022). Some of these actions include reduction of tumor weight in breast, lung and gastric cancer in animal and cell studies (Xu et al 2019). Dose dependent responses were seen in many of the reviewed cancer studies. Cytotoxic effects of *Mahonia* extracts were seen on breast, pharyngeal, and tongue cancer cells (Petruczynik et al 2019). Berberine is seen to work on multiple molecular pathways in cancer studies, with the key anti-inflammatory pathways seen in breast cancer being decreasing cytokines IL-1β, IL-6, TNF-α, and NF-kB (Karnam et al 2017). Small clinical studies show protection of lung damage from radiation as well as decreases in radiation induced intestinal side effects with berberine administration (Li et al 2010, Liu et al 2008). A study on multiple extracts of *Mahonia* from different parts of the plant showed many significant results. The root extract, the more traditionally used part of the plant, showed significant modulation of CD8+ T cells and IL-10. Interestingly the extract from green fruits of *Mahonia* showed a greater effect on TNF-α modulation as well as monocytes and β cells than the traditional root extract (Andreicut et al 2019).

Dermatological Conditions

Berberine shows antimicrobial effects against multiple skin bacteria including *Propionibacterium acnes* and *Staphylococcus* spp. (Clark et al 2017). Psoriasis immunopathology includes Th17 lymphocytes and their associated cytokines IL-17a, IL-17F, and IL-22 as well as TNF-α. A topical cream with 10% *Mahonia* extract has shown significant benefit in multiple clinical trials for psoriasis (Bernstein et al 2006, Janeczek et al 2018). The same topical preparation was significantly beneficial in one study on atopic dermatitis as well. Atopic dermatitis has a Th2 dominant immune profile with IL-4, IL-5, and IL-13 cytokines. (Janeczek et al 2018). This shows a wide breadth of immunomodulation capacity from topical applications.

Return to Traditional Usages – Gastrointestinal Actions and Microbiome Manipulation as Key Immunomodulatory Pathways

We can see that there is a growing evidence base of the multiple immunomodulatory actions of berberine. When we think of the role of herbal medicines in integrative treatment, we must consider their traditional uses in conjunction with the research base. In the case of berberine-containing herbs, this leads us to the digestive tract.

The impact of the gut on the immune system has become widely known in both medicine and the public in recent years. The balance of microorganisms in the gut stimulates or prevents inflammation through multiple pathways. The microbiota of the intestinal tract contains over 10^{14} microorganisms including bacteria, viruses, fungi, archaea, and protozoans (Lazar et al 2018). Gut microbiota influences the differentiation of T cells into Th1, Th2, Th17, and Treg cells. Secretory immunoglobulin A (IgA) is the first line of immune defense in the gut and its production is stimulated by commensal bacteria. In instances of dysbiosis, the gut's natural anti-inflammatory and protective immune actions become less effective. This creates room for further pathogenic colonization and opportunistic infections which further imbalance the gut immune system and contribute to inflammation (Beg et al 2011, Lazar et al 2018).

Organisms that increase the permeability of the intestines can create inflammatory conditions. Increased bacterial metabolites being passed through the gut immune cells leads to local and systemic inflammation. Disruption of lipopolysaccharides on gram-negative bacteria, through diet or other organisms, can also increase permeability and thus an increase in immune components like cytokines and macrophages (Al Bander et al 2020).

Berberine has specific action against multiple pathogens including *Escherichia coli* and *enterococci bacteri*a, and increases beneficial *Lactobacilli* and *Bifidobacteria* (Habtemariam 2016). It also remediates intestinal health by acting as an antimicrobial and by repairing the intestinal mucosa (Li et al 2020). Indeed, part of the success of *Hydrastis's* action on clearing infectious agents has been attributed to its ability to speed up healing of the affected tissue and restore host cell immune functions (Wood 2009). A 2020 study showed berberine's ability to up-

regulate tight junctions in the intestinal mucosa and suppressed fibrosis and cell infiltration in colon tissues (Li et al 2020).

Short-chain fatty acids (SCFAs), in particular butyrate, have been noted as an anti-inflammatory component in the gut. Well-balanced gut bacteria help break down complex carbohydrates into SCFAs and administration of butyrate improves symptoms in inflammatory GI diseases such as Crohn's (Al Bander et al 2020). SCFA production from certain bacteria, such as proprionate from *Phascolarctobacterium,* has been associated with NF-κB and with decreased levels of the acute phase reactant and inflammatory marker C-Reactive protein (Al Bander et al 2020). Oral administration of berberine in rat models has resulted in an increase in butyrate-producing bacteria (Yue et al 2019). This modulation of the intestinal microbial environment helps stabilize physiologic hypoxia in the intestines (Yue et al 2019). A human trial in diabetics showed berberine to change the population of 78 different species of gut microbiota and had improvements in clinical outcomes. In this study probiotics had minimal additional effect on microbiota changes compared to berberine (Zhang et al 2020). Along with the varied actions above, prevention of inflammatory cytokines in colitis with berberine has been well documented in murine models (Kawashima et al 2004, Lee et al 2010, Yan et al 2012, Zhang et al 2011, Zhou and Mineshita 2000). In a murine model of autoimmune uveitis, berberine-induced regulation of T-reg/Th17 cells was associated with changes in microbiota (Du et al 2020). This finding has been repeated in colitis models, with T reg/Th17 modulation being associated with changes in the bacterial balance (Cui et al 2018).

Environmental Considerations

With the current evidence base for berberine and berberine-containing herbs we are seeing it become a more commonly used therapy. Many of the ecological and supply issues of *Curcuma* can be applied to berberine-containing herbs. Using sustainably grown and wild crafted plants should always be considered when administering herbal medicines. Certain berberine-containing plants have specific ecological considerations.

Coptis is a challenging and low-yield plant to cultivate and is already in high demand. Its agriculture, as with most large-scale monocultures, is having detrimental effects on natural ecology. In the wild most *Coptis* spp are considered endangered or vulnerable and thus not recommended for wild crafting (Qin 2010).

Wild *Hydrastis* is a threatened plant in Canada and the United States. The cost of the cultivated plant has increased dramatically in recent years due to its more difficult cultivation and yield as well as demand (UPS 2022). Because of the many varieties of berberine-containing plants, choosing a locally sourced and affordable herb is still manageable. Wild *Mahonia* for example is very prolific in many parts of western North America and the rhizome can easily be sustainably harvested. With *Chelidonium* every part of the plant is used which often increases yield and facilities cultivation.

Conclusion

Berberine-containing herbs are found across the globe and have a multitude of actions. Through a combination of modern research and traditional usage we can see that berberine has multiple beneficial immune and inflammatory actions. Its ability to work on multiple pathways and immune cells can be applied to many different health conditions. One of the strongest actions of berberine is in the digestive tract, where it acts directly and in-directly on inflammation. Usage of berberine-containing herbs can improve inflammatory status through specific application to the conditions mentioned above and improve overall health through digestive and metabolic support. Because berberine-containing herbs can be found all over the world and in many different plant families and growing environments, they can be an effective and sustainable medicine choice.

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Hops (*Humulus lupulus*): An Overview of Clinical Applications

Heidi Fritz, MA, ND

Heidi Fritz, MA, ND
Bolton Naturopathic Clinic
Bolton, Ontario
info@boltonnaturopathic.ca

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Hops (*Humulus lupulus*): An Overview of Clinical Applications

Abstract

Hops (*Humulus lupulus*) has been used for millennia in the brewing of beer, imparting flavour and aroma to the beverage, as well as improving shelf life. Interest in hops as a medicine, however, did not gain momentum until the early 1800s. Modern investigation has revealed an array of constituents of interest, having impact across a broad number of important clinical indications. The prenylflavanoid xanthohumulol is a noted antioxidant and is receiving extensive preclinical interest as an anti-tumour agent. This molecule is capable of undergoing transformation by gut microbes giving rise to 8-prenylnaringenin, a powerful phytoestrogen heralded as delivering hops' impact to symptoms of menopause. The bitter acids of hops are principally credited with delivering effects related to sedation. The paper introduces clinicians to a versatile medicine, showcasing human level evidence of hops across an array of clinical indications that includes sleep, menopause, weight loss, cognition, mental health, allergy, and overactive bladder.

Introduction

Hops (*Humulus lupulus*) has been used for millennia in the brewing of beer, imparting flavour and aroma to the beverage, as well as improving shelf life. It is a climbing vine belonging to the *Cannabaceae* family. It is believed to be native to Eurasia, yet has been grown as various cultivars globally for centuries. Hops is perennial, growing aggressively from the end of April until the beginning of July, reaching heights of seven to eight meters under favourable conditions. Only female hops plants are commercially cultivated, as presence of male hops will turn the prized flowers of the female plants into valueless nuts (American Botanical Counsel 2022).

While the use of hops to improve flavour and shelf life of beer has been around for millennia, there was very little historical interest in medicinal applications of the plant. Paracelsus (1493-1541) seems to be the first scholar to mention hops as a medicine, citing its use as a digestive aid. Use of hops flowers as a bitter and a calming agent was first noted by Hecker in 1814 (American Botanical Counsel 2022).

The past several decades have seen a keen interest in a wide array of potential clinical applications of hops, and a large number of hops constituents have received considerable attention. Estrogenic principles of hops, notably 8-prenylnaringenin (8-PN) (and to a lesser extent 6-PN) have been extensively reviewed. 8-PN is widely considered the most potent phytoestrogen in the plant world (Calvo-Castro et al 2018, Chadwick et al 2006, Stulikova et al 2018). Prenylflavanoids, notably xanthohumulol, also receive keen research interest as both an antioxidant compound (Zugravu et al 2022) and potential antitumour agent (Girisa et al 2021, Jiang et al 2018, Zugravu et al 2022). Of tremendous interest is the ability of gut microbes to convert xanthohumulol to 8-PN (Possemiers et al 2006). Alpha and beta acids are antioxidants and are largely recognized for the sedating effects of hops (Ayabe et al 2020, Benkherouf et al 2020, Min et al 2021).

Many of the bitter constituents of hops degrade fairly rapidly, and as such hops is typically stored under refrigeration. Fifty to 70% of hops' bitter acids will degrade after only six months of storage (American Botanical Counsel 2022). An interesting observation among the various human trials of hops is that both fresh and matured, or oxidized, preparations are employed. Summaries of human trials below highlight whether the preparation is fresh or matured, when possible. It may be appropriate for a clinician to seek out both a fresh and matured version of the substance, depending which indication is principally sought from the intervention.

The objective of this review is to showcase human level evidence of clinical application of hops. Areas covered include sleep, menopause, weight loss, cognition, mental health, allergy, and overactive bladder.

Hops: Human Trials

A summary of human intervention trials utilizing hops can be found in **Table 1**. Of the 19 human trials presented, it should be noted that eight deliver hops in combination with other medicines. However, most of these trials (all but two) fall under the realm of hops as an agent to benefit sleep. Across all other indications, hops was principally used as a stand-alone ingredient.

Of the seven human trials reviewed for sleep, all but one (Franco et al 2012) examined hops in combination with other medicines. Most commonly, hops was combined with valerian. The one trial of hops as a stand-alone administered dealcoholized beer to female nurses working nightshift. While small, the trial was well controlled and showed significant positive impact using actigraphy as the principal endpoint measure (see **Table 1**) (Franco et al 2012). Another trial of note examining sleep compared valerian + hops to zolpidem showing improvement across both groups with no differences between groups (Maroo et al 2013). It is exciting to observe the gentle combination of valerian and hops achieving equivalent clinical outcome to a so called “Z-drug”.

Four trials are presented evaluating hops for the treatment of menopausal symptoms, three of which use hops as a stand-alone ingredient (Aghamiri et al 2016, Erkkola et al 2010, Heyerick et al 2006), while the fourth combines hops with soy (Kim et al 2021). The trials suffer from large positive placebo effects, yet some findings of significance are found favouring hops (see **Table 1**).

A very interesting pair of trials examines hops for weight loss and appetite suppression. Both trials use mature, or oxidized hops extracts, suggesting fresh versus mature preparations may be useful for different clinical applications. An RCT with 200 participants demonstrated reduced visceral fat and total fat area with CT used to confirm findings (Morimoto-Kobayashi et al 2016, Suzuki et al 2018). The second study found a significant impact on subjective reporting of hunger from participants following single administration of mature hops extract prior to a 24-hour fast (Walker et al 2019). **See Table 1.**

Two separate studies by the same research team demonstrated an important benefit of hops administration for various markers of cognition among healthy adults (Fukuda et al 2020A) and among adults with perceived subjective cognitive decline (Fukuda 2020B). Both studies used mature hops extracts. Two studies of hops are presented showing hops extract delivering significant benefit for mood, anxiety, and stress (Kyrou et al 2017, Ohara et al 2018). Also of interest is a trial showing impressive impact of a hops-containing beverage on allergic responses among individuals suffering from Japanese cedar pollinosis (Segawa et al 2007). Lastly, a large, well-controlled trial found impressive impact from a combination product containing hops on symptoms of overactive bladder, a condition notoriously difficult to treat (Gauruder-Burmester et al 2019). **See Table 1.**

Discussion

For a little over 15 years human trials have been evaluating hops for an array of clinical indications. Preclinical inquiry has demonstrated an unusually large number of molecules of interest, each with quite diverse targets in the human body. The most researched indication for hops, insomnia, typically evaluates hops in combination with other medicines, most commonly valerian. Quite reproducibly these combinations are proving successful in human study. The other areas of clinical inquiry are in need of further research, yet the available evidence certainly suggests use of hops for cognition, mental health, weight loss, and allergy is not without merit. Overactive bladder is notoriously difficult to treat, and the fact that a combination of natural medicines proved successful is encouraging. The combination included Uromedic pumpkin seed oil, *Rhus aromatica* bark extract, and *Humulus lupulus* cone extract. Reproduction of the study by Gauruder-Burmester and colleagues (2019) would be very welcomed.

None of the studies reviewed reported safety concerns of significance. An evaluation of herb-drug interaction administered a standardized hops extract to 16 peri- and postmenopausal women for two weeks, then challenged with a validated four-drug probe including tolbutamide, caffeine, dextromethorphan, and alprazolam. The paper found no significant impact of hops on several key P450 enzymes (CYP2C9, CYP1A2, CYP2D6, CYP3A4, and CYP3A5) (van Breemen et al 2020). Some animal evidence has demonstrated that hops can increase prolactin. A human trial gave a slow-cooked hops soup to men and women. The soup had no impact on prolactin in either group (Drugs and Lactation Database 2021).

Table 1. Human Intervention Trials of Hops

Methods	Outcomes	Reference
Sleep		
RCT in 101 participants with primary insomnia assigned to two capsules of olive oil (placebo) or two capsules of Cyclamax™ (260mg soya oil, 173mg Cade oil, 50mg Houblon (*Humulus lupulus* extract), and 6mg soya lecithin) two hours prior to bed for one month. Endpoint measures included LSEQ questionnaire, pre and post intervention actigraphic movement measurement, and urinary melatonin output.	Large positive impact to sleep quality, and modest improvement as measured by actigraphy, with no impact on urinary melatonin. However, the placebo group improved to a similar magnitude as the group receiving active treatment, and therefore there was no impact of the intervention relative to placebo.	Cornu et al 2010
Female nurses (N=17) working nights or rotating shifts assigned to receive 333ml 0% alcohol beer with dinner for 14 days. Participants served as their own controls.	Actigraphy demonstrated significant improvements for parameters of sleep latency and total activity during sleep, relative to baseline.	Franco et al 2012

Actigraphy pre and post intervention served as the primary endpoint measure.		
RCT in 91 participants with primary insomnia randomized to 10mg zolpidem or NSF-3™ (300mg valerian extract, 80mg passionflower extract, and 30mg hops extract) for two weeks. Assessments occurred at baseline, seven days, and upon study completion. ISI and ESSS served as main endpoint measures.	Both groups demonstrated significant improvement across many parameters of the ISI, notably total sleep time, sleep latency, number of nightly awakenings, and total ISI. ESSS was not significantly altered in either group. Differences between groups were not significant. Adverse events were reported 12 and 16 times with NSF-3™ and zolpidem, respectively, limited to concerns of drowsiness.	Maroo et al 2013
RCT in 171 participants randomized to placebo or Lactium™ (75mg per tablet, delivering 4.5g equivalent *Zizyphus*, 500mg equivalent *Humulus*, 52.5mg elemental magnesium as oxide, and 8.23mg vitamin B6) for two weeks. Primary outcome measure was sleep quality as assessed using the PSQI. Secondary outcomes included assessment of mood and anxiety, cognition, and stress reactivity.	PSQI improved significantly in both groups, with no between-group differences. Likewise, mood, anxiety, and stress improved significantly in both groups, with no between-group differences.	Scholey et al 2017
Patients (N=44) were administered 2ml of tincture (1:10 extract valerian + 1:12 extract hops in 61% ethanol) or placebo for two consecutive nights at a sleep clinic. Electrohypnography served as the principal endpoint measure.	The intervention significantly increased depth of sleep as well as total sleep time.	Dimpfel and Suter 2008
Patients were administered 500mg of valerian extract, valerian + 120mg hops extract, or placebo. Principle outcome measure was sleep latency.	Valerian + hops, yet not valerian alone, significantly reduced sleep latency.	Koetter et al 2007
Multicentre trial among 184 participants assigned to valerian-hops combination (187mg valerian extract + 41.9mg hops extract), or 25mg diphenhydramine or placebo for 28 days. Patient diaries, quality of life questionnaires, and	Individuals in the valerian-hops group, as well as the diphenhydramine group both experienced significant improvement in patient-rated insomnia score. Quality of life scores were also significantly	Morin et al 2005

polysomnography served as endpoint measures.	improved in the valerian-hops group. However, other measures of patient satisfaction, sleep latency, and polysomnography all failed to show outcomes of statistical significance.	
Menopause		
RCT of dried hops (flowering part) at 500mg per day versus placebo to 120 women for 12 weeks. Greene scale and number of hot flashes served as main endpoint measures.	The Greene scale significantly improved and the number of hot flashes was significantly reduced among participants receiving hops.	Aghamiri et al 2016
RCT of 67 menopausal women receiving placebo, or hops extract containing 100mcg or 250mcg of 8-prenylnaringenin. KI and patient questionnaire served as main endpoint measures.	All three groups achieved significant improvements in KI and subjective symptom reporting. The 100mcg per day dose performed superior to placebo at six weeks, yet not at 12 weeks.	Heyerick et al 2006
A soy and hops extract combination versus placebo was administered to 78 women experiencing menopausal symptoms for 12 weeks. KI (baseline greater than 20) served as the principal endpoint measure.	KI decreased a mean 20.61 points in the treatment group versus 14.80 in the placebo group ($p<0.05$). The treatment group also reported significant improvement relative to placebo in fatigue, paresthesia, arthralgia and myalgia, palpitation and vaginal dryness.	Kim et al 2021
A 16-week crossover trial in 36 women assigned to receive hops (75mg extract containing 100mg of 8-PN) or placebo for eight weeks. Subjects were then crossed over to receive the opposite treatment for the remaining eight weeks. KI and MRS served as principal endpoint measures.	At eight weeks, both hops and placebo significantly improved KI and MRS. After crossover, patients initially on hops now on placebo saw worsening of symptoms, whereas patients initially on placebo now on hops showed further improvements. By week 16, KI and MRS were significantly improved I the hops group relative to placebo group.	Erkkola et al 2010
Weight Loss		
RCT in 200 participants with BMI 25-30 randomized to placebo beverage or 350ml of test beverage containing mature hops extract providing 35mg of mature hop bitter acids for 12 weeks. CT of the	Significant reductions in visceral fat area were observed in the active group by week eight and remained significant at week 12. Total fat area was significantly	Morimoto-Kobayashi et al 2016 and Suzuki et al 2018

abdomen served as the primary endpoint measure.	reduced in the active group at week 12.	
Adult men (N=30) were participating in a 24 hour fast (6pm-6pm) one day per week for three weeks. Amarasate™, a bitter hops-based appetite suppressant was given at 500mg, 200mg, or placebo. Intervention was administered in divided doses 16 and 20 hours into each fast period. Visual analogue scales validated for subjective assessment of appetite served as the main endpoint measure.	Both doses of bitter hops extract produced significant reductions in hunger (>10%) relative to placebo. At no timepoint were outcomes different between the two doses of extract.	Walker et al 2019
Cognition		
MHBE 35mg per day versus placebo administered for 12 weeks to 60 healthy adults ages 45-64. A battery of neuropsychological questionnaires was conducted at six and 12 weeks.	MHBE significantly improved verbal fluency, subjective fatigue and anxiety. MHBE significantly reduced the Stroop test score.	Fukuda et al 2020A
Subjects (N=100) ages 45-69 with perceived subjective cognitive decline randomized to placebo or 35mg per day of MHBE for 12 weeks. A battery of eight validated scales were employed evaluating cognition, attention, stress and mood. Salivary assessment of B-endorphin, cortisol, chromogranin A and a-amylase was also performed.	SDMT and memory retrieval significantly improved in the group receiving MHBE. Salivary B-endorphin was significantly reduced in the group receiving MHBE. The authors conclude "MHBA improves cognitive function, attention, and mood state in older adults".	Fukuda et al 2020B
Mental Health		
Otherwise-healthy university students were screened using the DASS-21. Those with mild depression, anxiety and stress were enrolled. Crossover trial of four weeks each with a two-week washout. Hops dry extract at 400mg per day vs placebo.	DASS-21 significantly improved for mood, anxiety, and stress during treatment with hops versus placebo.	Kyrou et al 2017
Thirty minutes prior to an intentionally administered stress test (TSST), subjects consumed a test beverage containing 950ng of B-Eudesmol (an oxygenated sesquiterpene shown to affect autonomic nerve activity in animal	Following the TSST, participants consuming the test beverage had significantly lower levels of MHPG relative to participants consuming the placebo beverage.	Ohara et al 2018

models) or placebo beverage. Salivary 3-methoxy-4-hydroxyphenylglycol (MHPG) following the TSST served as the main endpoint measure (MHPG is a major breakdown product of noradrenaline)		
Allergy		
A beverage containing 100mg of hops water extract was administered versus a placebo beverage daily for 12 weeks during Japanese cedar pollinosis season to 39 participants. Symptom diary, blood sampling, and physical exam served as endpoint measures.	Participants receiving the hops water extract reported significantly fewer symptoms and reduced medication use. Physical exam demonstrated the hops group to have less nasal swelling, nasal discharge, and improved nasal colour and characteristics of nasal discharge. Eosinophil infiltration into nasal discharge was absent from hops-treated participants yet present in participants receiving the placebo beverage.	Segawa et al 2007
Overactive Bladder		
Open trial of Granu Fink femina (combination of Uromedic pumpkin seed oil, *Rhus aromatica* bark extract, and *Humulus lupulus* cone extract) administered to 117 women experiencing overactive bladder for 12 weeks. Symptom diary and quality of life questionnaires served as endpoint measures.	After 12 weeks of treatment, daytime urinary frequency significantly improved in 77/99 participants. Nighttime urinary frequency improved in 70/100 participants. Frequency of leakages and used pads significantly decreased from 0.9 and 2.0, respectively, at baseline to 0.4 and 1.4 respectively after 12 weeks of treatment.	Gauruder-Burmester et al 2019

Abbreviations

8-PN = 8-prenylnaringenin
DASS-21 = Depression anxiety stress scale-21
ESSS = Epworth sleepiness scale score
ISI = Insomnia severity index
KI = Kupperman Index
LSEQ = Leeds questionnaire evaluation sleep
MHBE = Matured hops bitter extract
MRS = Menopause rating scale
PSQI = Pittsburgh sleep quality index
RCT = Randomized controlled trial
SDMT = Symbol digit modalities test
STAI = State-trait anxiety inventory
TSST = Trier social stress test

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Effect of Consumption of Functional Foods Consisting of High-Oleic Canola Oil, Docosahexaenoic Acid and Barley β-glucan on Plasma Lipids, Blood Pressure, and Framingham Risk Score in Human Population with Metabolic Syndrome: A Randomized Crossover Trial

Anjalika Abraham, MSc,[1] and Peter JH Jones, PhD

*Corresponding author: Dr Peter JH Jones Nutritional Fundamentals for Health
351 Rue Joseph-Carrier, Vaudreuil-Dorion, QC J7V 5V5, Canada.
peterjones@nfh.ca

[1]Faculty of Agricultural and Food Sciences Human Nutritional Sciences, University of Manitoba

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Abstract

Functional foods such as high-oleic canola oil, omega-3 oils and barley β-glucan, have individually been shown to improve blood lipid profiles. The objective of the study was to test the synergistic effect of consuming a combination of novel foods with functional ingredients on blood lipid profile, blood pressure, and the Framingham risk score. A randomized single-blinded free-living crossover study design was used. The study involved thirty-five participants and consisted of four intervention phases, (1) muffins and cookies containing all-purpose flour and 50g/day of a blend of sunflower oil, safflower oil, and butter as a control oil; (2) barley flour with 4.36g/day of HMW-BBG and 50g/day of a blend of sunflower oil, safflower oil, and butter as control oil; (3) all-purpose flour, and 50g/day HOCO-DHA (85:15 DHA oil consists of 40% DHA) (DHA dosage 3g/day) and (4) a combination of barley flour with 4.36g/day, HMW-BBG and 50g/day of HOCO-DHA. The control oil used in the study represented a typical western dietary fat intake of four weeks duration, each intervention phase separated by a four-week washout period. Participants consumed test products of one muffin and two cookies. The combination of high-oleic canola oil, omega-3 oils and barley β-glucan showed an increase in high-density lipoprotein levels (HDL-C) ($P< 0.05$), a decrease in triglyceride levels (TG) ($P< 0.05$), decrease in very low-density lipoprotein levels (VLDL-C) ($P< 0.05$), total cholesterol to high-density lipoprotein ratio (TC:HDL-C) ($P< 0.05$) and diastolic blood pressure (DBP) ($P< 0.05$) at endpoint when compared to the control. No significant differences were observed for total cholesterol (TC) ($P< 0.05$), low-density lipoprotein level (LDL-C) ($P<0.05$), glucose level ($P< 0.05$) or systolic blood pressure (SBP) ($P< 0.05$). The combined treatment when compared to control at end point for the Framingham risk score was decreased for both males and females ($P< 0.05$). At endpoint, the high-oleic canola docosahexaenoic acid treatment showed an increase in high-density lipoprotein levels ($P< 0.05$), a decrease in triglyceride levels ($P< 0.05$), decrease in very low-density lipoprotein levels ($P< 0.05$), total cholesterol to high-density lipoprotein ratio ($P< 0.05$) and diastolic blood pressure ($P< 0.05$) when the high-oleic canola docosahexaenoic acid treatment was compared to the control. We conclude that the combination of high-oleic canola oil, omega-3 oils and barley β-glucan did not show a synergistic effect on the outcomes measured.

Introduction

Cardiovascular disease (CVD) risk factors are mainly associated with diets high in saturated fats and unhealthy lifestyle, leading to increased morbidity and mortality (Mertens et al 2017). Dietary portfolios using a combination of cholesterol-lowering foods offer additional positive outcomes compared to conventional dietary interventions in reducing serum cholesterol concentrations (AbuMweis et al 2010B, De Natale et al 2012, Jenkins et al 2011). However, the question remains whether the combination approach results in a synergistic or additive effect. Functional foods ingredients such as high-oleic canola oil (HOCO) (Gillingham et al 2011, Jones et al 2014, Senanayake et al 2014), omega-3 oils (Kelley et al 2007) and barley β-glucan (Behall et al 2004, Keenan et al 2007, Wang et al 2016) have been individually shown to improve blood lipid profiles. The canola multicentre trial (COMIT) study showed that a diet consisting of a combination high-oleic canola oil and docosahexaenoic acid (DHA) lowered high-density lipoprotein cholesterol (HDL-C) by 3.5%, triglyceride (TG) by 20%, systolic blood pressure (SBP) by 3.3% and showed a decrease in the ten-year Framingham risk score (FRS) (-19.0 ± 3.1%; $P<0.001$) when contrasted with other treatment diets, thereby lowering the CVD risk (Jones et al 2014, Senanayake et al 2014). The Omni-Heart Trial used a diet with 21% of energy from monounsaturated fatty acid (MUFA) and 10% from polyunsaturated fatty acids (PUFA). Results indicated a decrease in low-density lipoprotein cholesterol (LDL-C), blood pressure (BP) and CVD risk when compared to a higher carbohydrate diet (Swain et al 2008). The beneficial effects of n3 fatty acids on CVD risk reduction included modifying lipid profiles, reducing heart rate, reducing BP levels, improving endothelial function, improving arterial compliance, and alleviation of inflammatory responses (Bradberry and Hilleman 2013, Egert et al 2009, Skulas-Ray et al 2011). The omega-3-rich phase in the COMIT study at baseline showed a decrease in SBP by 3.3% when compared to other dietary treatments (Gillingham et al 2011, Senanayake et al 2014). A meta-analysis of 47 clinical studies and demonstrated the TG lowering effects of DHA on subjects with CVD when compared to the placebo group (Skulas-Ray et al 2011). The meta-analysis of eleven clinical trials showed that an intake of a minimum of 3g/d β-glucan soluble fibre from barley/oat grain products can reduce blood cholesterol concentrations (Summary 2012). In 1997, the US FDA approved a health claim for β-glucan soluble fibre from oats for reducing plasma cholesterol levels and risk of heart disease (US FDA 2006). AbuMweis et al (2010B) conducted a meta-analysis of randomized crossover trials to determine the effects of dietary consumption of BBG on serum cholesterol concentrations. Findings of the study indicated a 0.30mmol/L reduction in LDL-C levels after consuming BBG for four weeks when compared to control. The lipid-lowering effect of BBG suggested that an intake of 3g/day of BBG reduces serum blood concentrations of total and LDL-C levels. Following the positive outcome Health Canada in 2012 issued a health claim for BBG indicating that one gram which equals 35% of the recommended intake reduces blood cholesterol (AbuMweis et al 2010B). It is now recognized that the physicochemical properties of β-glucan, including molecular weight and viscosity, may play a role in the cholesterol-lowering effect of β-glucan (Lan-Pidhainy et al 2007, Vuksan et al 2011). Significant reductions were seen in serum TC and LDL-C levels with

3g/d high molecular weight barley β-glucan (HMW-BBG) in mildly hypercholesterolemic subjects (Behall et al 2004).

Taking into consideration the above-mentioned positive factors, the objective of this study was to test the effect of combining HOCO, DHA and HMW-BBG on blood lipid profiles, blood pressure and FRS in terms of observing a synergistic effect. This is the first study to test the effects of a combination of HOCO, DHA and HMW-BBG on CVD risk. It was hypothesized that this combined treatment for four weeks will lead to additional positive outcomes on blood lipids, blood pressure and FRS when compared to their consumption individually.

Materials and Methods

Study Design

The HOCO, DHA and HMW-BBG study was a randomized single blinded free-living crossover trial conducted at the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN). This was a seven-month study with four treatment phases, each 28 days in length separated by a four-week washout period. Prior to the commencement of the study as per protocol, each participant was required to be examined by a study physician. Participants then consumed one test muffin and two test cookies which were packed for consumption at home. Institutional research ethics boards reviewed and approved the study protocol. Trial registration can be viewed at www.clinicaltrials.gov with ID NCT-02091583 PROTOCOL NO: B2014:029. The trial protocol and study design has been published in Journal of Biomed Central. https://doi.org/10.1186/s13063-015-1014-5. All participants provided written informed consent as prescribed by the research ethics board.

Eligibility Criteria and Subject Selection

Study advertisements used local media including newspapers, the official website of RCFFN, university campus, and a pre-existing database of participants. The eligibility criteria included age: 18-70 years, body mass index: >25kg/m^2, waist circumference: men: >94cm, women > 80cm. Individuals with at least two of the following criteria were accepted into the study: i) TG >1.7mmol/L; ii) HDL-C <1mmol/L for male and <1.3mmol/L for female; iii) fasting glucose >5.6mmol/L iv) LDL -C >2.7mmol/L and v) blood pressure >130/100. The exclusion criteria included: (i) participants consuming lipid lowering medications or nutritional supplements known to affect blood lipids, or having any dietary restrictions that would prevent them from consuming the study diet for 28 days, (ii) participants with current or history of any diseases and disorders that could interfere with fat absorption, (iii) participants planning to conceive during the study period, (iv) smoking or people consuming more than one alcoholic drink per day or history of alcoholism or drug dependence, or (v) people having consumed any experimental medication within one month before screening or as concomitant medications. Twelve-hour fasting blood samples were collected on days one, two, 29 and 30 to test lipid profiles. The main outcome measures were serum lipid variables, including TC, LDL-C, HDL-C, TG, and VLDL-C concentrations. Blood samples were centrifuged at 3000rpm for 20min at 4°C, and were

separated to yield serum, plasma, and RBC, and then stored at -80°C until analysis. Monitoring of blood pressure, body weight, and waist circumference was done at the beginning and at the end of each treatment period. Three measurements were taken and the average of the three was calculated. Whole body dual-energy x-ray absorptiometry (DEXA) scan (Lunar Prodigy Advance, Lunar Corp., Madison WI) was conducted to determine changes in body fat composition at the end of each treatment phase.

Study Diets

Thirty-five participants were randomly assigned to each of the four treatments from January 2015 to November 2015. The randomized and recruited participants consumed one muffin along with the breakfast at the centre and the two cookies were packed for consumption later in the day. There were two flavours of muffins (pumpkin spice and vanilla) and three flavours of cookies (cocoa, lemon and ginger). The flavours of the muffins and cookies were provided in rotation with equal numbers of days per flavour during each treatment period. For the weekend, the muffin and cookies were packed and given on Friday. There was minimal control over the background diets of the participants. The dietitian provided individualized dietary recommendations based on the Canada's Food Guide according to his/her energy requirements before the commencement of the study. Almost 50% of the treatments consumed were under supervision to ensure optimal compliance. Participants were instructed to restrict their intake of fish or seafood products. A three-day food record was collected at the beginning and end of each treatment phase. Participants were strongly advised to maintain consistency in their dietary intake, physical activity patterns as well as body weight during the experimental period. Recruited participants were randomly assigned to one of the treatment sequences using a Latin square design. The nutrient compositions of the muffins and cookies for the different treatments of the study are listed in **Tables 1 and 2** respectively. The four treatments were as follows: (1) muffins and cookies containing all-purpose flour and 50g/day of a blend of sunflower oil, safflower oil, and butter as a control oil. (2) barley flour with 4.36g/day of HMW-BBG and 50g/day of a blend of sunflower oil, safflower oil, and butter as a control oil. (3) all-purpose flour and 50g/day HOCO-DHA (85:15 DHA oil consists of 40% DHA) (DHA dosage 3g/day) and (4) combination of barley flour with 4.36g/day, HMW-BBG and 50g/day of HOCO-DHA. The control oil used in the study represented a typical western dietary fat intake. The control treatment was composed largely of saturated fat with substantial levels of n-6 linoleic acid as seen commonly in North American diets. Dosages for HOCO-DHA and HMW-BBG were determined based on previous clinical studies done by our research group (AbuMweiss et al 2010B, Gillingham et al 2011). The most common procedure of analysis is to perform a separate calculation based on target effect sizes for each of the interventions compared with their respective controls.

The set of four treatments was analyzed using a 2x2 factorial design, which represents two factors (oil and flour) each at two levels. The two levels of oil were control oil and HOCO-DHA, and the two levels of flour were control flour and HMW-BBG. The effects investigated by this

design were the two main effects of the two factors and their interaction effect. A factorial design is an efficient way for exploring the effects of oil and flour in the treatments we used in our study. This trial design is useful to detect a link between factors while reducing the possibility of experimental error or confounding variables. It also highlights the relationships between factors, allowing the effects of manipulating a single factor to be isolated and analyzed singly. The purposes of a factorial design are (i) to attain information on the average effects of all the factors from a single experiment of moderate size, (ii) to expand the basis of inferences on one factor by testing it under varied conditions of others, and (iii) to assess the way the effects of factors interact with one another. Here is an example that used a 2x2 factor experiment to study the effects of two factors theobromine and cocoa on blood lipids. Their analysis showed a significant main effect of theobromine on HDL-C ($P<0.05$) but not cocoa, and no significant interaction effect (Neufingerl et al 2013). The HOPE-3 trial is another example using a 2x2 factorial trial showed the significant effect of the rouvastatin group on lowering risk of CVD events ($P<0.05$) but not candesartan + hydrochlorothiazide and no interaction effect was seen (Yusuf et al 2016). These two examples of factorial analysis illustrate how the effects of individual factors and their interaction can be studied.

Muffin and cookie recipes were developed in the Agriculture and Agri-Food Canada laboratories and at the RCFFN metabolic kitchen. Alberta Barley Commission provided the barley grain (cultivar CDC Rattan) and milled this grain into whole grain flour at the Canadian International Grains Institute, Winnipeg. The cultivar CDC Rattan was selected based on the β-glucan content and because CDC Rattan is a popular food barley variety in western Canada. All-purpose flour (Robin Hood) was purchased from the local supermarket. Richardson Oilseed Limited provided the HOCO. The DHA oil was purchased from DSM Nutritional Products (Ayr, ON, Canada). Macronutrients, including protein, carbohydrates, and fibre content of the foods, were analyzed at the Agriculture and Agri-Food Canada laboratories, RCFFN, Winnipeg, Canada. Total fat and fatty acid profiles of the foods were measured at the RCFFN laboratories Winnipeg, MB, Canada. The macronutrient contents of treatment foods were similar between different treatments and there were no differences in nutritional values between different flavours of muffins or cookies.

Sample Collection and Analysis

Twelve-hour fasting blood samples were collected on days one, two, 29 and 30 of each intervention phase. Participants were advised to fast for 12 hours and not consume any alcoholic beverages for at least 48 hours before blood collection. Participants were instructed to refrain from engaging in intense physical activity for at least 24 hours before each visit. To determine participant compliance, on the days of blood collection, a medical questionnaire was filled to record their 12-hour diet, 48-hour alcohol consumption, and 24-hour physical activity patterns. Blood samples were centrifuged at 3000rpm for 20 minutes at 4°C. Aliquots of samples yielded serum, plasma EDTA and heparin, and red blood cells (RBC) EDTA and heparin and stored at −80°C until analysis.

Lipid Profile and Framingham Risk Score Analysis

Analysis of plasma samples for lipid profiles including TC, HDL-C, TG, LDL-C, VLDL-C and glucose levels were performed utilizing robotized enzymatic routines on Vitros 350 Chemistry System (Ortho-Clinical and Diagnostics, Johnson and Johnson). For the analyses, 0.5ml of plasma heparin sample was used to measure the lipid profile. The system was initially calibrated for each parameter and then measured using Calibrator Kit 1 (glucose), Calibrator Kit 2 (TC, TG, LDL-C) and Calibrator Kit 25 (HDL-C). This was followed by using Performance Verifier 1 and 2 to check for the value of each parameter in the given ranges as prescribed online. The Performance Verifier was run every eight hours to ensure accuracy in results. Serum LDL-C concentrations were estimated using the Friedewald formula (LDL-C = TC - HDL-C - TG/5) (Friedewald et al 1972). FRS 10-year CHD risk score was estimated by FRS online calculator based on age, sex, SBP, TC, and HDL-C values of each participant at the end of each dietary phase (D'Agostino et al 2008). Blood pressure was measured using a digital sphygmomanometer. Readings were averaged at 10, 13 and 16 minutes.

Statistical Analysis

Statistical analysis of the effects of the treatments on the lipid profile, Framingham risk on serum cholesterol concentration was tested using SAS (version 9.4; SAS Institute, Inc., Cary, NC, USA). A power calculation indicated that a minimum of 35 participants was necessary to detect an interaction effect in LDL-C concentrations of 0.43mMol/L with a standard deviation of 0.13mMol/L in a 2x2 factorial full design, 2-sided testing with alpha = 0.05 and power = 0.8. To allow for a dropout rate of 20%, the desired sample size was increased to 28. The mixed model procedures in SAS (Proc mixed in version 9.4) were used with treatment, sequence, phase, and gender considered as fixed effects, whereas subject/participant was added as a random effect in the model. Treatment effects in the model were analyzed as a factorial set with the two factors, Oil type and Flour type and their interaction. The purpose of analyzing treatment effects as a factorial is to dissect the factorial effects of oil with two levels (Control and HOCO-DHA), flour with two levels (Control and BBG) and their interaction. The normality of distribution of data was determined by a Shapiro-Wilk test, and the non-normal variables were normalized by identifying the outliers from the studentized residual effect before comparisons with other treatments. Results for treatments in the text and figures are expressed as least-squares means and their standard errors from the model described above, unless stated otherwise. Multiple comparison of treatment means was carried out using Tukey's test with a type I error of 0.05.

Results

Baseline Characteristics and Treatment Differences

Thirty subjects completed all four treatment periods. Seven participants were excluded from the study. Four dropped out during the study due to low tolerance to the test products. One was assessed as non-compliant during the study and the remaining two individuals were found to be non-compliant based on lab and statistical analysis and were excluded. Twenty-eight

participants' data were finally analyzed (**Figure 1**). No statistically significant differences were observed for subjects across treatments, age, BMI, TC, glucose, LDL-C, HDL-C, TG, body weight, waist circumference, SBP and DBP at baseline as seen in **Table 3**.

Effect of Treatment on Lipid Profile and Blood Pressure

At endpoint, the HOCO, DHA treatment showed an increase in HDL-C ($P< 0.05$) and a decrease in TG ($P< 0.05$) when compared to control. We also observed a decrease in VLDL-C ($P< 0.05$), TC:HDL-C ($P< 0.05$) and DBP ($P< 0.05$) in the HOCO, DHA treatment when compared to the control. The combination of HOCO, DHA and HMW-BBG showed an increase in HDL-C ($P< 0.05$) and a decrease in TG ($P< 0.05$) when compared to control. The combination of HOCO, DHA and HMW-BBG also showed a decrease in VLDL-C ($P< 0.05$), TC:HDL-C ($P< 0.05$) and DBP ($P< 0.05$) at endpoint when compared to the control. No significant differences were observed for TC, LDL-C, glucose level and SBP **Table 4.** Between treatments, the HOCO DHA treatment was significantly different ($P<0.05$) when compared to HMW-BBG treatment for TG, HDL-C, VLDL-C, TC:HDL-C and DBP. Similarly, the combined HOCO, DHA and HMW-BBG treatment was significantly different ($P<0.05$) when compared to HMW-BBG treatment for TG, HDL-C, VLDL-C, TC:HDL-C and DBP. At endpoint, in a 2x2 factor analysis, there was a significant main effect on the HDL-C, VLDL-C, TC:HDL-C, TG and DBP, shown for HOCO DHA oil factor ($P< 0.05$) but not for the HMW-BBG factor. There was no significant interaction effect between HOCO, DHA and HMW-BBG on any of the lipid parameters ($P>0.05$) as shown in **Table 5**. At endpoint the combination of HOCO, DHA and HMW-BBG was also able to show an overall decrease in FRS for both males and females when compared to the control ($P<0.05$) **(Figure 2)**. Between treatments there were no significant differences.

Discussion

The purpose of this study was to evaluate the effect of combining functional ingredients HOCO, DHA and HMW-BBG on blood lipid profiles and FRS in a population with MetS. After a four-week treatment period the combination of HOCO, DHA and HMW-BBG improved HDL-C, lowered TC: HDL-C ratio, TG, VLDL-C and DBP as well as showed an overall decrease in FRS for both males and females when compared to the control. The overall significant decrease in cholesterol FRS for the combined treatment was observed because of increased HDL-C concentrations and a decrease in blood pressure. However, cholesterol FRS changes in other and between treatments were not significantly different. Based on previous literature, decreases in blood pressure are identified to be related to changes in vascular function, indicating that blood pressure measurements were conducted in a consistent manner. Understandably, the HOCO-DHA treatment also showed an increase in HDL-C, decrease in TC: HDL-C ratio, TG, VLDL-C and DBP when compared to control. Some of the results of the study are consistent with the COMIT trial, where the HOCO-DHA was the only diet that increased HDL-C, as well as produced the greatest reduction in TG and lowered SBP and DBP when compared with the other diets (Gillingham et al 2011). The TC: HDL-C ratio is also a strong predictor of CVD risk.

Owing to the disproportion between the atherogenic cholesterol carrier and the protective lipoproteins cholesterol carrier, individuals with a higher TC: HDL-C ratio have greater CVD risk (Lutjohann et al 2002). The HMW-BBG individual treatment showed no significant effects when compared to control. The AHA guidelines recommend 2-4g/d fish oil supplement daily for individuals at risk for CVD (Lutjohann et al 2002). Effect of dose response studies showed that 3g/d of DHA was capable of lowering TG levels whereas dosages as low as 0.85g/d did not. The dose of DHA (3g/d) used in the current study was based on the recommended range for TG lowering, to evaluate individuals with CVD risk factors (Skulas-Ray et al 2019). Serum LDL-C tended to decrease after the consumption of the combination of HOCO, DHA and HMW-BBG, however, this reduction was not statistically significant. Omega-3 fatty acid studies have shown that the TG lowering effect occurs with an increase in LDL-C concentration (Teunissen et al 2001). The theory is that the intake of DHA based on previous literature could have resulted in an increase in LDL-C while lowering TG. At the same time the effect of HMW-BBG could have resulted in lowering of LDL-C. The increase in LDL-C could have thus blunted the effect of HMW-BBG, as seen in previously conducted trials. As a result of this push-pull mechanism, these combined actions may have led to no change observed for LDL-C. The main objective of this study was to determine the efficacy of functional ingredients of HOCO, DHA and HMW-BBG combined into a single food format and observe whether a synergistic effect exists on blood lipids and FRS. Our findings show that the significant effects of the combined HOCO, DHA and HMW-BBG treatment seen in the lipid profile and FRS were not over and above the individual treatment effect sizes, indicating that despite the positive outcomes of combining HOCO, DHA and HMW-BBG they had no overall synergistic impacts.

In conclusion, despite the overall positive outcome, the combination of HOCO, DHA and HMW-BBG had no significant synergistic effect on circulating cholesterol levels or FRS during the 28-day intervention period. The evidence clearly outlines the need to find an acceptable dosage of oil to match with 3g/d HMW-BBG in order to achieve the targeted goals or preliminary screening of the right genotype that could provide better clinical outcomes.

Acknowledgements and Statement of Conflict of Interest

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Figures and Tables

Table 1
Nutritional Composition of Study Muffins

Nutritional Component Measurements	Control oil + Control flour	Control oil + HMW BBG	HOCO-DHA + Control flour	HOCO-DHA + HMW BBG
Weight (g)	112	108	113	109
Energy (kcal/kJ)[a]	379/1586	383/1602	379/1586	383/1602
Total carbohydrates (g)[a]	32.7	36.6	33	36.59
Total proteins (g)[a]	8.34	8.79	8	9
Total fibre (g)[a]	0.36	5.77	0.36	5.77
β-glucan (g)[a]	0	2.18	0	2.18
Total fat (% weight)	22.5	22.9	24	23
SFA (%)	33.08	35.24	13.2	14.77
MUFA (%)	27.93	27.16	56.71	52.44
PUFA (%)	37.96	38.16	30.04	33.79
n-3 PUFA (%)	0.86	1.01	5.59	5.64
n-6 PUFA (%)	37.11	37.60	24.93	27.15

Note: *DHA* docosahexaenoic acid, *HOCO* high-oleic acid canola oil, *MUFA* monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid, *SFA* saturated fatty acid. Values presented are based on values of the ingredients. Muffins were made with two different flavours: vanilla and spice. Values presented are the average of both the flavours, and there were no major differences between flavours for the measurement. Butter, sunflower and safflower oils (55:20:25) (control oil; 50gm/d) and all-purpose flour. Barley β- glucan (4.3gm/d) and butter sunflower and safflower oils (55:20:25) (control oil; 50gm/d). High-oleic Canola DHA (85:15) (50gm/d, 3gm/d) and butter sunflower and safflower oils (55:20:25) (control oil; 50gm/d) and all-purpose flour. High-oleic Canola DHA (85:15) (50gm/d, 3gm/d) and barley β- glucan (4.3gm/d)

Table 2
Nutritional Composition of Study Cookies

Nutritional Component Measurements	Control oil + Control flour	Control oil + HMW BBG	HOCO-DHA + Control flour	HOCO-DHA + HMW BBG
Weight (g)	53	50	52	52
Energy (kcal/kJ)[a]	213/891	221/924	213/891	221/924
Total carbohydrates (g)[a]	23	25	23	25
Total proteins (g)[a]	4	4	4	4
Total fibre (g)[a]	0.46	3.17	0.46	3.17
β-glucan (g)[a]	0	1.09	0	1.09
Total fat (% weight)	24	24	24	23
SFA (%)	33.75	35.4	12.50	12.6
MUFA (%)	31.08	29.0	63.4	64.23
PUFA (%)	35.2	35.7	24.13	23.20
n-3 PUFA (%)	0.48	0.61	6.44	6.28
n-6 PUFA (%)	34.7	34.91	17.5	16.9

Note: DHA docosahexaenoic acid, HOCO high-oleic acid canola oil, MUFA monounsaturated fatty acid, PUFA polyunsaturated fatty acid, SFA saturated fatty acid. Values presented are based on values of the ingredients. Cookies were made with three different flavours: cocoa, lemon and ginger. Values presented are the average of both the flavours, and there were no major differences between flavours for the measurement. Butter, sunflower and safflower oils (55:20:25) (control oil; 50gm/d) and all-purpose flour. Barley β- glucan (4.3gm/d) and butter sunflower and safflower oils (55:20:25) (control oil; 50gm/d). High-oleic Canola DHA (85:15) (50gm/d, 3gm/d) and butter sunflower and safflower oils (55:20:25) (control oil; 50gm/d) and all-purpose flour. High-oleic Canola DHA (85:15) (50gm/d, 3gm/d) and barley β- glucan (4.3gm/d).

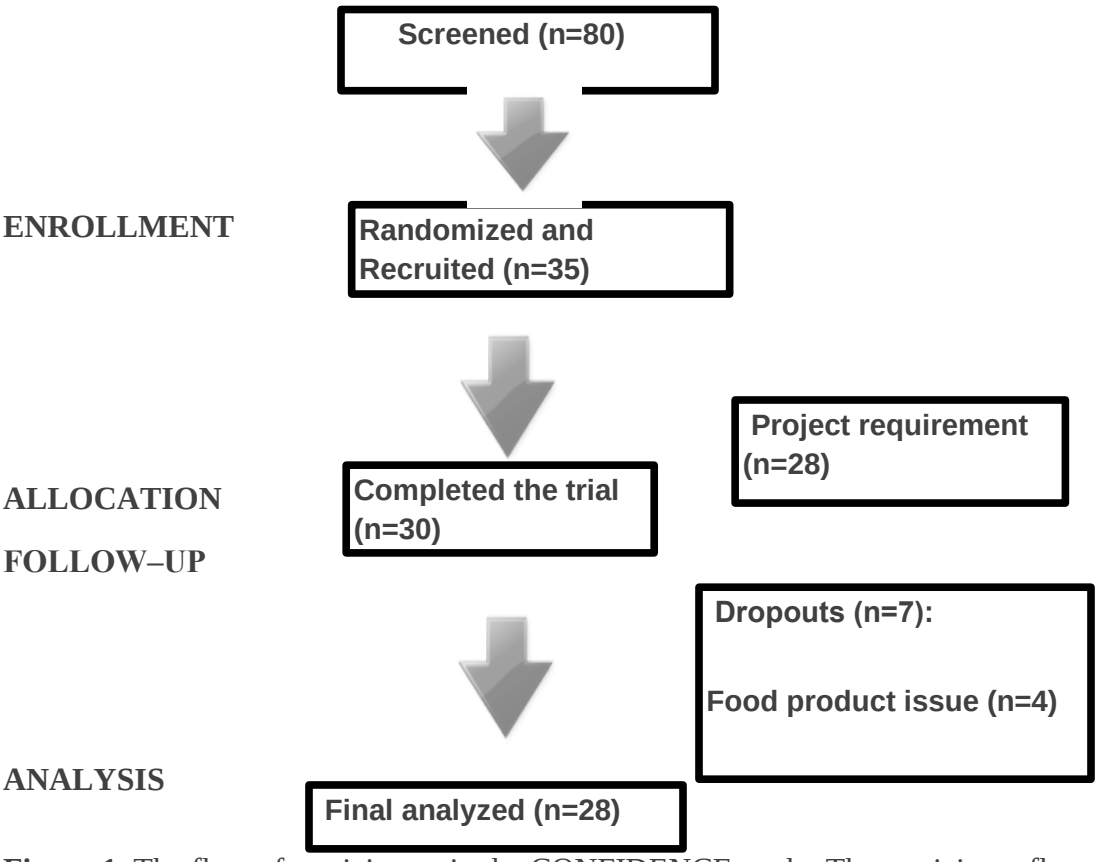

Figure 1. The flow of participants in the CONFIDENCE study. The participant flow through each step of the recruitment, screening and study protocol process at the RCFFN.

Table 3
Baseline Characteristics of Study Participants

	Control oil + Control flour	Control oil + HMW BBG	HOCO-DHA + Control flour	HOCO-DHA + HMW BBG
MALE[a] (n)	12	12	12	12
FEMALE[a] (n)	17	17	17	17
AGE[a] (years)	50 ± 2	50 ± 2	50 ± 2	50 ± 2
Bmi[a] (kg/m^2)	31.6 ± 5.2	31.6 ± 5.1	31.5 ± 5.1	31.4 ± 5.0
Body Weight[a] (kg)	89.3 ± 17.1	89.1 ± 17.2	88.8 ± 16.7	88.5 ± 17.1
TC[b] (mmol/L)	5.30± 0.7	5.32 ± 0.72	5.38 ± 0.72	5.36 ± 0.8
LDL-C[b] (mmol/L)	3.31 ± 0.66	3.23 ± 0.56	3.33 ±0.56	3.34 ± 0.67
HDL-C[b] (mmol/L)	1.19 ± 0.21	1.17 ±0.22	1.21 ± 0.22	1.17 ± 0.2
TG[b] (mmol/L)	1.69 ± 0.74	1.96 ± 1.0	1.76 ± 1.0	1.82 ± 0.84
VLDL[b] (mmol/L)	29.9 ± 13.0	34.7 ± 17.0	31.37 ± 12.0	32.3 ± 14.9
Glucose[b](mmol/L)	5.52 ± 0.6	5.43 ± 0.72	5.47 ± 0.72	5.60 ± 0.7
SBP[b] (mmHg)	119 ± 12.4	120 ± 14.0	118 ± 13.9	120 ± 15.4
DBP[b] (mmHg)	79.6 ± 8.1	81.1 ± 8.6	80.0 ± 8.6	79.7 ± 7.1

Note: Values are representative of subsample of 28 participants (RCFFN) for individual treatment phases with means and their standard deviations.[a] Anthropometric measurements.[b] Lipid profile. TC-Total Cholesterol, LDL-C- Low-density lipoprotein cholesterol, DHDL- Direct High-density lipoprotein, TG- Triglycerides, SBP- Systolic blood pressure, DBP- Diastolic blood pressure.

Table 4
Between Treatment Comparisons of Blood Lipid Profile, Blood Pressure Levels

	Control oil + Control flour	Control oil + HMW BBG	HOCO-DHA + Control flour	HOCO-DHA+ HMW BBG
TC[b] (mmol/L)	5.40± 0.14	5.26 ± 0.00	5.39 ± 0.13	5.20 ± 0.15
LDL-C[b] (mmol/L)	3.35 ± 0.12	3.25 ± 0.13	3.35 ±0.13	3.19 ± 0.13
HDL-C[b] (mmol/L)	1.20 ± 0.03[a]	1.18 ±0.04[a]	1.30 ± 0.05[b]	1.31 ± 0.05[bc]
TG[b] (mmol/L)	1.79 ± 0.13[a]	1.84 ± 0.13 [a]	1.58 ± 0.16[b]	1.56 ± 0.13[bc]
VLDL[b] (mmol/L)	32.4 ± 2.5[a]	30.7 ± 2.1[a]	28.5 ± 2.8[b]	27.3 ± 2.3[bc]
TC: HDL-C[b] (mmol/L)	4.54 ±0.48 [a]	4.53± 0.15 [a]	4.23 ± 0.15[b]	4.09 ± 0.14[bc]
Glucose[b] (mmol/L)	5.49 ± 0.10	5.40 ± 0.11	5.71 ± 0.17	5.63 ± 0.15
SBP[b] (mmHg)	122 ± 3.55	118 ± 2.75	119 ± 3.13	120 ± 2.70
DBP[b] (mmHg)	81.0 ± 1.81[a]	80.0± 1.50 [a]	78.2 ± 1.80[b]	78.1 ± 1.70[bc]
FRS*	7.77±0.49 [a]	7.17± 0.40 [a]	7.04± 0.49 [a]	6.87± 0.50[bc]

Note: Values are representative of subsample of 28 participants (RCFFN) for individual treatment phases with means and their standard errors at endpoint.[a] Anthropometric measurements.[b] Lipid profile. TC-Total Cholesterol, LDL-C- Low-density lipoprotein cholesterol, DHDL- Direct High-density lipoprotein, TG- Triglycerides, SBP- Systolic blood pressure, DBP- Diastolic blood pressure. Data at endpoint measurement. Means in a column for a given factor or interaction which do not show a common letter are significantly different (P<0.05). A mixed-model ANOVA and post hoc Tukey's test was used. Butter, sunflower and safflower oils (55:20:25) (control oil; 50gm/d) and all-purpose flour. Barley β-glucan (4.3g/d) and butter sunflower and safflower oils (55:20:25) (control oil; 50g/d). High-oleic Canola DHA (85:15) (50g/d, 3g/d) and Butter sunflower and safflower oils (55:20:25) (control oil; 50g/d) and all-purpose flour. High-oleic Canola DHA (85:15) (50g/d, 3g/d) and barley β-glucan (4.3g/d). *For FRS the HOCODHA and HMW-BBG treatment is only significantly different from the control oil+ control flour treatment.

Table 5
Factorial Effects and Their Significance for Blood Lipid Profiles and Blood Pressure Levels (P values)

	HMW-BBG	HOCO-DHA	HOCO-DHA + HMW BBG
TC[b] (mmol/L)	0.09	0.90	0.29
LDL-C[b] (mmol/L)	0.06	0.63	0.61
HDL-C[b] (mmol/L)	0.5	<0.001**	0.55
TG[b] (mmol/L)	0.48	0.01**	0.81
VLDL[b] (mmol/L)	0.38	0.00**	0.82
TC:HDL[b] (mmol/L)	0.22	<0.001**	0.26
Glucose[b] (mmol/L)	0.91	0.10	0.60
SBP[b] (mmHg)	0.27	0.80	0.17
DBP[b] (mmHg)	0.61	0.04**	0.72

Note: Values are representative of subsample of 28 participants (RCFFN) for individual treatment phases. [a]Anthropometric measurements. [b] Lipid profile. TC-Total Cholesterol, LDL-C-Low-density lipoprotein cholesterol, DHDL- Direct High-density lipoprotein, TG- Triglycerides, SBP- Systolic blood pressure, DBP- Diastolic blood pressure. Data at endpoint measurement. Means in a column for a given factor or interaction which show** are significant (P<0.05). A mixed-model ANOVA and post hoc Tukey's test was used. Butter, sunflower and safflower oils (55:20:25) (control oil; 50gm/d) and all-purpose flour. Barley β- glucan (4.3gm/d) and butter sunflower and safflower oils (55:20:25) (control oil; 50gm/d). High-oleic Canola DHA (85:15) (50gm/d, 3gm/d) and Butter sunflower and safflower oils (55:20:25) (control oil; 50gm/d) and all-purpose flour. High-oleic Canola DHA (85:15) (50gm/d, 3gm/d) and barley β-glucan (4.3gm/d).

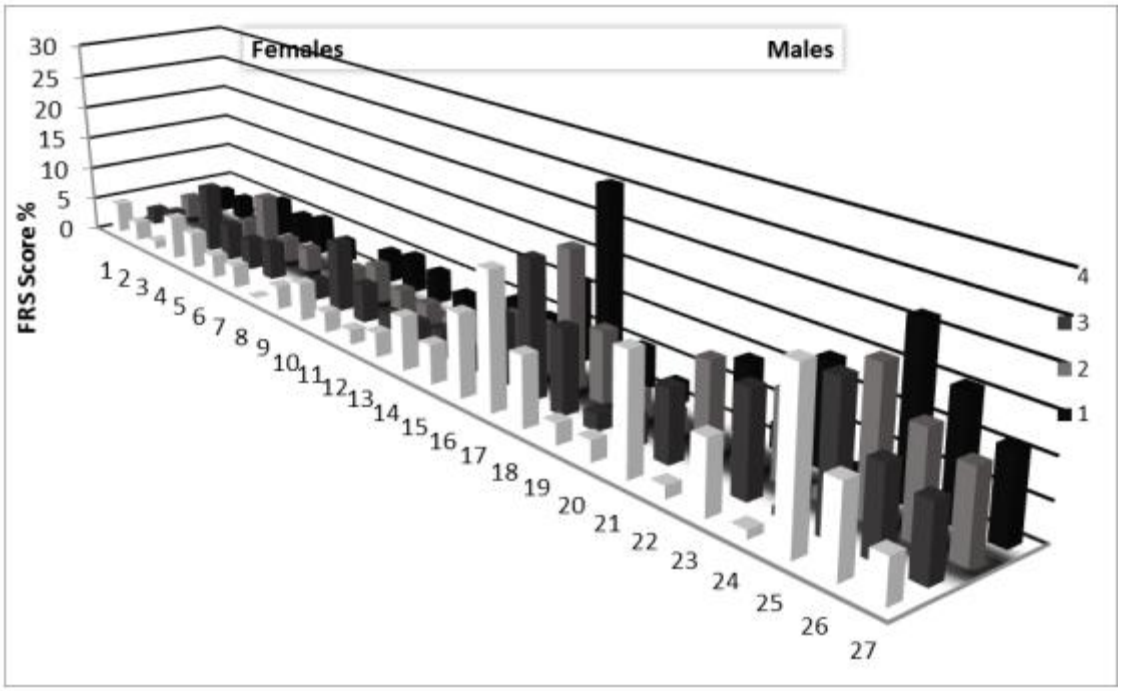

Figure 2: Individual changes for both males and females in Framingham risk score at endpoint. Numbers 1-27 indicate individual participant changes for all four treatments. FRS is calculated for ages 30-100 years, as one participant is below 30 years total number of participants is 27. Females are 1-16 and Males are from 17-27. The legends in numbered format indicate: Treatment 1: Butter, sunflower and safflower oils (55:20:25) (control oil; 50g/d) and all- purpose flour. Treatment 2: Barley β-glucan (4.3gm/d) and butter sunflower and safflower oils (55:20:25) (control oil; 50g/d). Treatment 3: High-oleic Canola DHA (85:15) (50g/d, 3g/d) and butter sunflower and safflower oils (55:20:25) (control oil; 50gm/d) and all-purpose flour. Treatment 4: High-oleic Canola DHA (85:15) (50g/d, 3g/d) and Barley β-glucan (4.3g/d)