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Publisher's Letter

Staying Positive

CJNM is off to an exciting start! Still in the early days, the Journal is growing an impressive following. Reflecting on the success of the Journal has been humbling. Dr. Rouchotas and the team are on the pulse of where the industry is going and how it is evolving. The quality of the Journal is being elevated to an amazing level. I am very eager to see what further steps the team is able to make, and am confident you, the reader, will continue to receive information immediately implementable in your practice and professional development.

Another note I want to touch on is the glass full theory. The pandemic although challenging has allowed us to do things that we thought we had no time for or could not do. With less travel and conferences, we have more time to spend on building our businesses, cooking new foods, spending quality time with our family and adapting to the new norm. I think it is important that we reflect on the good that has come out of this pandemic. It's clear a lot of people are feeling warn down and frustrated with what's going on. I have been doing my best to be a positive voice during these times, reminding people of what has been gained as opposed to focusing on what has been lost, and I believe it is making a difference for the people I interact with. I encourage our readers to try to do the same. Remind patients, vendors, your family of all the positive things the new norm has brought to them.

Happy holidays stay safe!

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Editor's Letter

Winter is Coming…

Seems much of Canada, while treated to the mildest fall I can remember, was quickly thrust into an early and angry winter. My heart goes out to all the people in western Canada impacted by the major storms we watched unfold with horror. I hope your recovery is speedy and complete.

The Holiday season is upon us, and the focus in our home is the Christmas tree. Not to mention not so subtle reminders from the kids as to what they are hoping to get from Santa. I wish you all a safe, restful, and joyous Holiday!

CJNM wraps up 2021 with an excellent lineup of contributions. With tremendous excitement CJNM presents its first original research article! The team behind the human trial reads as a who's who of academia. A truly impressive multicentre trial evaluating diets of differing fat composition and their impact on glucose control, with further evaluation of various genetic markers and how they influenced trial outcomes. CJNM aspires to be a resource for original clinical research, and the trial presented should succeed in attracting future high-quality original research submissions.

The lineup of review articles is must-read stuff! Neil McKinney does a tremendous job of showcasing the basis for consideration of cannabidiol in cancer care. Odessa Gill and Erin Valente introduce a simple, non-invasive liver scan with impressive sensitivity and specificity for detecting liver fibrosis. The test can easily be rolled out in outpatient integrative healthcare practice. Tori Hudson and Sarah Tindall deliver an excellent review of ginger with a focus on women's health. Several applications backed with human level evidence are certainly news to me! Daniella Perri delivers an eloquent and detailed review of mechanisms through which vitamin D achieves outcomes of immunomodulation. I am privileged to provide a review establishing safety of low- hyperforin extracts of St John's wort with all classes of prescription medications.

Happy reading and stay warm! The CJNM team looks forward to delivering Volume 2, Issue 1 in March of 2022.

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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.

Canadian Journal of Naturopathic Medicine

Cannabidiol (CBD) in Cancer Care

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The Author declares no conflicts of interest

Cannabidiol (CBD) in Cancer Care

Abstract

Cannabidiol (CBD) is a medically active but non-psychotropic constituent of *Cannabis spp.* It is a modulator of the intrinsic endocannbinoid system, which has significant regulatory and homeostatic functions in several important systems in the human body. Of particular interest is the impact CBD may have on the immune system, inflammation, anxiety, pain, and neuronal injury. Emerging evidence suggests these properties of CBD may be a valuable adjunct to the standard of care in oncology. It may reduce harm from chemotherapy and radiation therapy with no reduction in therapeutic efficacy. It may moderate graft-versus-host disease after stem cell transplants and moderate the formation of cancer stem cells involved in cancer progression, spread, treatment resistance, and relapses. CBD may even directly suppress cancer cells via mitochondrial-dependent apoptosis and autophagy mechanisms.

Hemp-derived medicinal CBD

Hemp is a type of cannabis used for many centuries for fiber, fuel, oil, and seed. Canvas and other fabric, rope, and birdseed are examples of industrial products from this plant. Hemp is *not* marijuana. The content of the psychotropic (mind-bending) cannabinoid THC, that gets people high, is little to none in hemp strains of cannabis. Hemp derivatives carry none of the illicit drug stigma attached to marijuana. Some have argued CBD can convert into psychotropic THC, but this is not proving to be clinically relevant in humans (Golombek et al 2020).

Due to low content of the narcotic cannabinoid THC, hemp seed products (eg, hemp seed oil), without the cannabinoid-rich resin from the flowering tops, are explicitly excluded by the definition of cannabis in the UN 1961 single convention on narcotic drugs, are generally regarded as safe, and are legal to market in many countries (United Nations 2021).

Cannabidiol, or CBD, is the most useful cannabinoid in hemp. CBD acts through our endocannabinoid system, built into almost every organ and function in the human body. We make chemicals similar to the cannabinoids from these plants. We are all using our own built-in human endocannabioids to adjust our critical systems including metabolism, digestion, blood pressure, appetite, body temperature, bone density, synthesis of fats, fertility, moods, anxiety, arousal, pain signaling, immune function, and the inflammatory response. The plant versions of cannabinoids turn out to just be external sources of natural biochemicals that adjust this internal human endocannabinoid system. CBD in cannabis inhibits the enzymatic clearance of our natural human endocannabinoid anandamide (AEA), allowing levels to rise. CBD stimulates the release of our other natural endocannabinoid two-acylglycerol, aka two-arachidonyl glycerol (2 AG), also increasing its abundance in tissues (Britch et al 2021).

CBD binds to the two types of cannabinoid receptors found in the brain and nervous system, the gut, on immune cells, and elsewhere, called CB1-R and CB2-R. Since it acts on receptors in the brain, and increases feel-good substances, it is important to note that it does not have any narcotic or stupefying effect. In fact, CBD may blunt some of the intoxicating effects of THC. So, while CBD does act on the brain and nervous system and is therefore psychoactive, it is not psychotropic (Crippa et al 2018).

CBD has a low affinity for the CB1 and CB2 receptors *per se*, but it blocks the fatty-acid binding protein that transports our innate endocannabinoids to be hydrolyzed, which prolongs the activation of the CB1 receptor. It also modulates other receptors such as the 5-hydroxytryptamine (5-HT1A) serotonin receptor and the peroxisome proliferator-activated receptor γ (PPAR-γ). Some of its pharmacological effects are also caused by it binding to other cell surface G-protein receptors (GPRs). For example, the anticonvulsant, anti-spasmodic, anxiolytic, anti-emetic, anti-depressant, analgesic and neuro-protective effects of CBD are thought to be conferred by several GPRs on nerve cells (Kiskova et al 2019).

CBD protects brain cells called astrocytes from injury and speeds recovery of nerves from trauma. It is a significant antioxidant, neuro-protective, and anti-inflammatory in various models of brain insults and injuries. In addition, CBD has been shown to reduce pain, spasticity, and seizures, to reduce peripheral and central nervous system inflammation, and to modulate blood circulation and metabolism. It is anxiolytic (treats anxiety) and reduces psychosis. Importantly, CBD is also cytotoxic to cancer cells, meaning it directly kills them (Kozela et al 2017).

Can CBD help cancer patients better tolerate therapy?

Cancer is a frightening disease, and the therapies that can cure it can also be scary. Surgery, chemotherapy, receptor targeted drugs, and radiation all have consequences that can induce anxiety, and in some cases, sleeplessness. CBD has become quite widely accepted as useful for anxiety and insomnia. However, it must be understood that human research trials have so far been limited in number and scope, and so health claims are still classed as "unproven". That being said, there is a great deal of "pre-clinical evidence" based on studies in cells in petri dishes (*in vitro*) and in rodents (*in vivo*) pointing to the potential of CBD, and other cannabinoids, to be of benefit in many aspects of cancer care. While clinical experience is not research evidence, patient uptake of CBD has arisen from a "grassroots" (no pun intended) sharing of experience, and clinicians are seeing many patients who find consistent and significant reasons to use CBD for common ailments. CBD is showing a very favorable benefit versus risk of harm ratio. There is a clear need to get on with proper clinical trials to move from anecdote to evidence.

The first widespread acceptance of cannabinoids in oncology was for chemotherapy-induced nausea and vomiting (Schussel et al 2018). Cannabinoids have been accepted in palliative care for emesis and pain (Sledzinski et al 2018). There is some "reasonable amount of evidence" for cannabis use in nausea and vomiting, loss of appetite, and pain as a supplement to first-line treatments, and data suggestive of possible use to treat chemotherapy-induced peripheral neuropathy, gastrointestinal distress, and sleep disorders. Scant yet more controversial, evidence exists in regard to cannabis for cancer and cancer treatment-related cognitive impairment, anxiety, depression, and fatigue (Kleckner et al 2019).

CBD was found to reduce neuropathic pain from Taxane chemotherapy via the 5-hydroxytryptamine (5-HT1A) serotonin receptor with no cognitive impairment or conditioned rewarding effects (Ward et al 2014). It was neuroactive but not psychotropic. It is clear, however, that for severe pain THC combined with CBD is ideal (Abrams et al 2011, Martin-Sanchez et al 2009). An animal study suggests that CBD is protective against Paclitaxel-induced neurotoxicity mediated in part by the 5-HT1A receptor system, and is able to suppress inflammatory and neuropathic pain (Xiong et al 2012).

Two synthetic THC drugs are FDA approved for use in cancer: nabilone (chemo nausea and vomiting, and sleep) and dronabinol (CINV, cachexia in AIDS). Sativex (nabiximols), a combination of THC and CBD as an oral spray, was approved in Canada and the European Union for neuropathic (nerve) pain in multiple sclerosis (MS) and intractable cancer pain.

Clinical studies have shown that Sativex has beneficial effects on spasticity, mobility, bladder function, and pain in MS patients. While researchers report it is well tolerated, patients tend to stop using it due to high cost and mouth irritation with long term use. Many patients want some of these benefits, but for a variety of reasons cannot or will not use THC, the narcotic cannabinoid. Fortunately, CBD acts on nausea and vomiting (N/V, emesis) in a biphasic manner, being quite effective in moderate doses, though it is capable of exacerbating N/V in high doses. The anti-nausea effect of CBD is mediated in part by the 5-HT1A receptor system (Likar and Nahler 2017).

CBD as Epidiolex (GW Pharmaceuticals) was recently approved by the U.S. Food and Drug Administration (FDA) to treat rare forms of epilepsy.

Cannabinoids may improve radiation and chemotherapies in pancreatic and lung cancer (Yasmin-Karim et al 2018). CBD enhances radiation impact on glioblastoma cells (Ivanov et al 2017) and reduces expression of immune-suppressive PD-L1 gene/protein/cell surface expression (Ivanov et al 2019), suggesting it might also synergize with the new classes of immune checkpoint inhibiting drugs.

CBD sensitizes cancer cells to chemotherapy drugs by inhibiting exosome and microvesicle release from cancer cells, trapping more drug in the cancer cells (Kosgodage et al 2018). Extracellular vesicles (EVs) are key mediators for cellular communication through the transfer of proteins and genetic material. Cancers, such as the aggressive brain cancer glioblastoma multiforme (GBM), use EV release for drug-efflux (bailing out drugs out to spare cancer cells from lethal levels), pro-oncogenic (cancer growth stimulation) signaling, invasion, and immune-suppression. EV-inhibitors have been shown to increase sensitivity of cancer cells to chemotherapy. Cannabidiol is such an EV-modulator in GBM cells exposed to the chemotherapy drug temozolomide (TMZ). Compared to controls, CBD-treated cells released EVs containing lower levels of pro-oncogenic miR21 and increased levels of anti-oncogenic miR126; these effects were greater than with TMZ alone. In addition, prohibitin (PHB), a multifunctional protein with mitochondrial protective properties and chemo-resistant functions, was reduced in GBM cells by CBD. CBD may, via modulation of EVs and PHB, act as an adjunct to enhance TMZ treatment efficacy in GBM (Kosgodage et al 2019).

CBD interacts favorably with chemotherapy drugs in part due to its anti-inflammatory and anti-oxidant properties. CBD reduces cardio-myopathy from Doxorubicin (Hao et al 2015), the key issue which limits the lifetime exposure to this class of drugs that is tolerable to the heart. CBD overcomes resistance to Oxaliplatin in colorectal cancer cells via autophagy induced by the overproduction of ROS through mitochondrial dysfunction (Jeong et al 2019A).

Transient receptor potential vanilloid type-2 (TRPV2) is an ion channel that is triggered by agonists like cannabidiol (CBD). Via TRPV2 receptors, CBD increases cancer cell (e.g., leukemia) uptake of chemo drugs such as temozolomide, doxorubicin, and carmustine (Pellati et al 2018). CBD improved chemotherapeutic drugs cytotoxic effects on uterine (endometrial)

cancer cells, also linked to TRPV2 over-expression. (Marinelli et al 2020). Activating TRPV2 channels with CBD increased hepatocellular (liver) cancer cell uptake of doxorubicin (Neumann-Raizel et al 2019). TRVP2 is a useful target in triple-negative breast cancer (TNBC), an aggressive cancer with few therapy options other than chemotherapy. TRPV2 activation by CBD significantly increased doxorubicin chemo drug uptake and increased apoptosis (programmed cell death) in TNBC cells (Elbaz et al 2016). This chemo-enhancing effect was more recently confirmed with CBD and doxorubicin in an *in vivo* mouse model of triple-negative breast cancer (Laezza et al 2020).

Bone marrow or peripheral stem cell transplantation is a life-saving therapy for cancers such as leukemia and some lymphomas. The patient gets rid of the old, damaged marrow that made their immune cells, and a new immune system is installed. The new immune cells can kill remaining cancer cells, but they can also damage the host's healthy cells and tissues. Graft-versus-host-disease (GVHD) is a major obstacle to successful allogeneic hematopoietic cell transplantation (alloHCT). Cannabidiol has potent anti-inflammatory and immune-suppressive properties. In a phase II study of patients with acute leukemia or myelodysplastic syndrome, none of the patients developed acute GVHD while consuming CBD. Lower grade reactions were significantly reduced, well beyond the standard of care procedures for GVHD (Yeshurun et al 2015).

Can CBD actually kill cancer cells on its own?

Cancer patients can get comfort and care from ingesting CBD, and it appears it can enhance the "standard of care" medical and radiation oncology therapeutics. However, those therapies do not save all cancer patients from harm or even eventual death from the disease. There is developing evidence that CBD may actually shrink tumors and extend life.

There are literally hundreds of mutations in cancer cells, a plethora of growth stimulating factors, and metabolic issues that drive the uncontrolled growth of cells that is cancer. Based on the preliminary evidence in various models, it appears that cannabinoids target key signaling pathways involved in all the hallmarks of cancer (Pyszniak et al 2016). There are many other drivers of cancer growth and spread we apparently can target with CBD. Studies demonstrate anti-proliferative, pro-apoptotic, cytotoxic, anti-invasive, anti-antiangiogenic, anti-inflammatory, and immunomodulatory properties of CBD. It has reduced initiation, progression, and metastasis in several different types of cancer.

The most important single focus of cancer research right now is the issue of *cancer stem cells (CSCs).* Stem cells are involved in wound healing, tissue repair, and are used as a medical therapy for many conditions. However, stem cells can be corrupted to produce cancer cells, and cancer cells can adapt and develop stem cell properties or "stemness". These corrupted cells are the only ones inside a tumor that can invade into adjacent tissues or spaces, including into lymph vessels and blood vessels. They are mobile! Cancerized stem cells or CSCs are the only cells able to freely spread into distant organs or metastasize. They can colonize and set a up a new tumor – they are "tumorigenic". This is typically when cancer is called stage 4 and incurable.

They are the only cells in a tumor that can reproduce infinitely – they are "immortal" (Nassar and Blanpain 2016). So, to be clear, CSCs are responsible for the most malignant aspects of the disease, and turn a dangerous situation into a life-threatening one.

Cancer cells acquire a malignant "stemness" through a process called the epithelial–mesenchymal transition (EMT). During the EMT, epithelial markers (including E-cadherin) are down-regulated and mesenchymal markers are upregulated. CBD downregulating expression of receptor CB1 in breast cancer cells, blocking migration and progression of the IL-1-induced signaling pathway IL-1/IL-1RI/-catenin, the primary driver of EMT. Cannabidiol localized E-cadherin and catenin at the adherens junctions. It also prevented catenin nuclear translocation. This reduced cell viability, tumor progression, and spread (Garcia-Morales et al 2020). CBD combined with THC inhibits EMT in non-small cell lung cancer (NSCLC) cells (Milian et al 2020). CBD kills prostate cancer cells *in vitro*, and significantly reduced melanoma tumor growth and increased survival time and quality of life *in vivo* (De Petrocellis et al 2013).

One of the most dangerous brain cancers, glioblastoma multiforme (GBM), has a high resistance to our most aggressive therapies and very high rates of reoccurrence. This is partly related to the presence of glioma stem-like cells (GSCs). GSCs express cannabinoid receptors, CB1 and CB2, as well as other components of the endocannabinoid system. Cannabinoid agonists altered the expression of genes involved in stem cell proliferation and differentiation (Laezza et al 2020).

CBD also modulates marrow derived stem cells (MDSCs), innate myeloid cells that possess the ability to control immune responses. These tend to show up when tumors become very large, crowded, low in oxygen, heavily fementative, and are vigorously pumping acid out into their surrounding microenvironment (milieu). Contrary to popular belief that cancer cells are acidic, they are actually slightly alkaline compared to non-cancerous cells of the same type. Fermentative metabolism creates masses of acidic lactate which is pushed by proton efflux pumps out into the extracellular milieu/microenvironment. This is why alkalizing therapies do not do more than palliate the patient, with little to no impact on the cancer growth (Hao et al 2018). When the inflammation around and in the tumors exceeds the capacity of the local immune controls, the big marrow stem cells are brought in to try to put out the fire, but cancer is "the wound that will not heal". Controlling inflammation at time of surgery and thereafter is key to managing stem cells, invasion, metastasis, and thus to prevent crossing the threshold from curable to incurable.

Stem cells can be brought under control by inducing cell differentiation – forcing them to specialize, and settle down into a normal function. Anti-inflammatory and antioxidant, CBD via TRV2 receptors influences differentiation of glioma stem cells (Pellati et al 2018). In tumors derived from glioma stem cells (GSCs), CBD inhibited disease progression. CBD-dependent production of ROS was accompanied by reduction in glutathione (GSH) and GSH-related enzymes (McAllister et al 2015). Cancer cells acquire, generate, and store (sequester) unusual levels of GSH to protect themselves from stresses of crowding, acid waste, low oxygen, and

more. Many oncology therapies work at least in part by depleting tumor glutathione. CBD-dependent production of ROS was accompanied by reduction in glutathione (GSH) and GSH-related enzymes (McAllister et al 2015). The activities of glutathione reductase and glutathione peroxidase were significantly decreased in those treated with CBD, inducing apoptosis (Cerretani et al 2020).

There are three ways cells die. Necrosis is an abrupt and messy process caused by severe circumstances such as burns. Autophagy is a cell self-digesting, often just recycling parts, but in some cases recycling the whole cell. This is a commonly used target in treating brain cancers. Apoptosis or programmed cell death is a more orderly process, used in healthy tissues to remove old or dead cells, recycle them, and to trigger a replacement to be made. Normal cells can only double a limited number of times before this apoptosis program removes them.

Apoptosis is fundamentally how chemotherapy drugs and radiation, two foundations of oncology, actually kill cancer cells. Cancer cells have a way of shutting off this kill switch, so they can continue to double and grow without limit.

Any therapy that forces a cancer cell to restore this safety system will result in cancer cells recognizing it is time to go, and to go quietly.

CBD induces endoplasmic reticulum and mitochondrial membrane stress by inhibiting the AKT/mammalian target of rapamycin (mTOR) signaling, inducing apoptosis in breast cancer cells. CBD led to an interaction between PPAR, mTOR, and cyclin D1 to the advantage of apoptosis induction in breast cancer. Similar induction of apoptosis is also seen in lung, prostate, brain, and colorectal cancer cells (Kis et al 2019).

Mitochondria are key to apoptosis. These are the little metabolic organelles inside cells where fuel is burned with oxygen for energy or fermented (no oxygen) to make cell materials. As cancers progress, cells end up with fewer functioning mitochondria, and the loss of and damage to mitochondria has a direct and linear relationship to the rate of growth and spread. CB1 receptors are present on the mitochondrial membrane, where activation can directly control cellular respiration, energy production, and generation of reactive oxygen species (Benard et al 2012). CBD modulates voltage-dependent anion channel on mitochondria to induce cancer cell death (Rimmerman et al 2013).

CBD reduced invasiveness and metastasis in cells of aggressive breast cancer through its interaction with Id-1, an inhibitor of basic helix-loop-helix transcription factors. Id-1 is a key regulator of the metastatic potential of breast and additional cancers (McAllister et al 2011). CBD inhibits human breast cancer cell proliferation and invasion through differential modulation of the extracellular signal-regulated kinase (ERK) and reactive oxygen species (ROS) pathways, and that both pathways lead to down-regulation of Id-1 expression and up-regulation of the pro-differentiation factor Id-2 (McAllister et al 2011).

CBD treatment perturbs the function of the mitochondria as suggested by loss of mitochondrial membrane potential and release of cytochrome c. The cumulative cellular stress achieves activation of multiple intrinsic and extrinsic caspases, the enzymes that dissolve cells undergoing apoptosis. Importantly, CBD treatment inhibited tumor progression and induced apoptosis *in vivo*. The ability of CBD to inhibit cancer cell viability/proliferation has been reversed in the presence of antagonists for CB2, TRPV1, TRPM8, COX-2, and PPRγ. CBD produced a concentration-dependent increase in calcium leading to alterations in mitochondrial membrane potential, production of ROS, and ultimately cytotoxicity (Massi et al 2013). CBD induces apoptosis in cancer cells via increased mitochondrial release of Smac, which turns off XIAP, an inhibitor of caspases (Jeong et al 2019A, Zhang et al 2019).

Mitochondrial RNA (miRNA) dysregulation in cancer cells is overcome by CBD, inducing apoptosis in neuroblastoma cells (Alharris et al 2019).

Mitochondria are central to cancer survival and progression, in particular due to their central role in calcium signal control, which is altered in cancer. Critically, CBD has been shown to modulate mitochondrial function, and thus, calcium signaling. (Kosgodage et al 2019). Calcium signaling remodelling in cancers has consequential impact on key events such as proliferation, invasion, and sensitivity to cell death. Specific calcium signaling pathways have also now been identified as playing important roles in the establishment and maintenance of multidrug resistance and the tumor microenvironment (Monteith et al 2017). Mitochondria-associated membranes (MAMs) are critical hubs in signal transduction involved in cancer onset and progression. Perturbation of calcium homeostasis at the MAMs in cancer cells is correlated with impaired cell proliferation and death (Danese et al 2017). CBD potently increases mitochondrial calcium allowing stable transition pores through which the apoptosis caspases can pass. Altered calcium status in mitochondria is a hallmark of cancer and a key to cancer cell resistance to apoptosis. CBD directly targets mitochondria and alters their capacity to handle calcium ions (Ca^{2+}). At lethal concentrations, CBD causes mitochondrial calcium ion overload, stable mitochondrial transition pore formation, and cell death in acute lymphoblastic leukemia of T lineage (T-ALL), but not resting healthy T cells. This effect may be helpful during chemotherapy for leukemia. CBD also induces autophagy (Olivas-Aguirre et al 2019). Based on the same apoptosis and autophagy mechanisms, CBD inhibits breast cancer, gliomas, lung cancer, and leukemia (Massi et al 2013). CBD is an agonist (stimulant) modulating TRPV2 channel passage of essential ions such as Na^{+} and Ca^{++}, impacting glioblastoma cell proliferation (Ryan et al 2009). This also kills malignant brain oligodendrocytes (Mato et al 2010). CBD-mediated calcium regulation via mitochondria can restore apoptosis (Pumroy et al 2019).

Recall that autophagy is cell death by self-digestion. CBD led to endoplasmic reticulum stress, inhibition of the AKT/mTOR pathway, and up-regulation of autophagy-mediated cell death in breast, pancreatic, and melanoma cell lines. CBD induced autophagic cell death in part through the initial induction of the ROS sensor AMP-activated protein kinase (AMPK) (McAllister et al

2015). CBD induces autophagy in colorectal cancer cells, again, by the overproduction of ROS through induced mitochondrial dysfunction (Jeong et al 2019B).

Recent studies have shown that CBD reduces cancer cell viability in many cancer types such as neuroblastoma, glioblastoma, melanoma, leukemia, colorectal, breast, lung, and prostate cancer. Many *in vitro* and *in vivo* experiments have demonstrated that cannabinoids have potential to inhibit angiogenesis (blood vessel growth into tumors) and metastasis. CBD is connected to downregulation of an expression of Id-1, an inhibitor of basic helix–loop–helix transcription factors, which has been shown to be a key regulator of the metastatic potential of breast cancer. It has also been demonstrated that CBD can lead to a decrease in lung tumor cell invasion and metastasis via the mechanism relied on for the upregulation of the intercellular adhesion molecule 1 (ICAM-1). Cannabidiol has anti-cancer activity, acting as potent antagonists of TRPM8 receptors (McAllister et al 2015).

CBD inhibits EGF/EGFR in aggressive forms of breast cancer, including TNBC. CBD significantly inhibits epidermal growth factor (EGF)-induced proliferation and chemotaxis (mobility to invade or metastasize) of breast cancer cells *in vitro*. Further studies revealed that CBD inhibits EGF induced activation of EGFR, ERK, AKT, and NF-kB signaling pathways as well as MMP2 and MMP9 secretion. CBD inhibits tumor growth and metastasis in *in vivo* mouse model systems. Analysis of molecular mechanisms revealed that CBD significantly inhibits the recruitment of tumor-associated macrophages in primary tumor stroma and secondary lung metastases (Elbaz et al 2015). Macrophages are large immune cells which can make up to 50% of the volume of some breast tumors. They are associated with tumor growth and spread, including by engulfing but not digesting cancer cells, forming fusion hybrid cells, which carry tumorigenic malignant cells to distant places. Immune cells can transfer or apply their special power to move through tissues to the cancer cells.

CBD is a novel immune modulator via CB1 and CB2 receptors and transient receptor potential vanilloid 1. CBD inhibits critical activators of the Janus kinase/signal transducer and activator of transcription signaling pathway, as well as the nucleotide-binding oligomerization domain-like receptor signaling pathway. This decreases pro-inflammatory cytokine production. Furthermore, CBD protects against cellular damage incurred during immune responses by modulating adenosine signaling. These immune-suppressive effects of CBD mark it as a potentially effective immune modulatory therapeutic (Peyravian et al 2020).

CBD acts on the immune system through transient receptor potential V1 (TRPV1), also known as the vanilloid receptor. CBD's effects on neuroinflammation and colitis have been shown to be mediated by peroxisome proliferator-activated receptor gamma (PPAR-c). CBD has a biphasic action on the immune system, including activation of immune regulatory cells. T cell-produced IFN-c is a critical target of CBD suppression. CBD's ability to suppress transcription factors such as NFAT, AP-1, and NF-kB likely accounts for its widespread suppression of many immune cytokines. CBD is anti-inflammatory via inhibition of fatty acid amide hydrolase

(FAAH), as well as via some cytokines and chemokines such as IL-4, IL-5, IL-13, and eotaxin (Nichols and Kaplan 2020).

It is very difficult to do long term studies proving any single agent prevents cancer. However, in colorectal adenocarcinoma, there is a link to the formation of aberrant crypt foci (ACF) and progression of polyps to cancer. CBD reduced ACF formation and reduced CRC tumor volume in experimental models (Orrego-Gonzales et al 2020).

Summary

Cannabidiol is a medicine of great value in a variety of medical conditions, including sleep, anxiety, nerve pain, inflammation, nausea, and epilepsy. It is becoming an accepted palliative for these conditions in cancer patients.

There is pre-clinical evidence showing great potential for the use of CBD as a support for the standard oncology therapeutics – surgery, radiation, chemotherapy, and immunotherapy drugs. It may reduce harms and increase the beneficial outcomes beyond the current “standard of care”. It is emerging as potent tool in controlling graft-versus host disease post-stem cell transplant, and prevention as well as treatment of neuropathy from chemotherapeutic drugs.

There are many mechanistic arguments that can be made that CBD has actions that may kill cancer cells via cytotoxicity, apoptosis, autophagy, and immune regulation.

CBD appears to act very favorably to suppress the corrupted cancer stem cells which resist therapy, invade, metastasize, and can reproduce infinitely. CBD may even act as a cancer preventative.

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FibroScan as a Simple Non-Invasive Screening Tool in Predicting Fibrosis in Non-Alcoholic Fatty Liver Disease Patients

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Abstract

The use of FibroScan in a clinical setting has been well established. Numerous studies demonstrate the efficacy of FibroScan as a screening tool in non-alcoholic fatty liver disease (NAFLD) patients. This non-invasive device is a valuable tool in a naturopathic practice to help identify NAFLD patients and those with suspected liver disease. The article will showcase human evidence assessing sensitivity and specificity of this important clinical tool. It is hoped the paper encourages more naturopathic doctors to make this simple, safe, and cost-effective tool available to their patients.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a condition in which there is an accumulation of excess fat in hepatocytes in people who consume little or no alcohol. The prevalence of NAFLD is estimated to be 20 to 40% of the population (Eddowes et al 2019). Many people with NAFLD are asymptomatic and physical examination is unremarkable. Therefore, early detection and treatment can lead to the prevention of more progressive forms of liver disease.

Using non-invasive testing to detect fatty infiltration and identify the stage of liver disease is imperative in a clinical setting as liver-related health concerns are on the rise. Transient elastography (TE) is a simple and safe ultrasound-based technique to measure liver scarring. FibroScan is a device that utilizes this technique to assess liver 'hardness' or 'stiffness'. This translates to the degree of fibrosis. Assessment of fibrosis provides information about prognosis and allows practitioners to determine treatment. The FibroScan is a powerful tool in naturopathic practice as it provides practitioners with an easy method to evaluate liver health. With chronic liver disease increasing, the FibroScan device can be used as a tool in the management of patients with chronic liver disease and in investigating suspected liver disease. Test results can estimate the degree of liver illness and guide future management and collaboration with other health care professionals.

What is a FibroScan?

FibroScan is a non-invasive device that assesses for stiffness of the liver using TE. Fibrous tissue is harder than normal liver tissue, therefore the degree of fibrosis can be inferred from the liver hardness. This stiffness is evaluated by measuring the velocity of a vibration wave that is generated on the skin (Castera et al 2008). The measurement happens on the surface of the abdomen over the liver. This vibration wave is also referred to as a shear wave. The shear wave velocity measures the time the vibration wave takes to travel to a particular depth in the liver (Castera et al 2008). The result is a measurement of liver stiffness, hence liver stiffness measurement (LSM). In addition, by measuring the ultrasonic attenuation of the echo wave, hepatic steatosis can be quantified. This is termed the controlled attenuation parameter (CAP). A minimum of 10 valid readings are performed to improve test reliability (Castera et al 2008). The LSM varies between 2.5 and 75 kPa (Castera et al 2008). Healthy patients will have a LSM less than 7.0 kPa (median reading 5.3 kPa) (Castera et al 2008). The CAP score ranges from 100 to 400 dB/m and different ranges result in different stages of steatosis. The FibroScan result is then used to determine the fibrosis score. This score ranges from F0 to F4 (see Table 1). The interpretation of the fibrosis stage should be completed in addition to other clinical tools such as ultrasound and serum liver function tests.

Table 1: Scoring System for Fibrosis Stage

F0	No scarring
F1	Mild fibrosis
F2	Moderate fibrosis
F3	Severe fibrosis
F4	Cirrhosis or advanced fibrosis

The FibroScan is an easy method to evaluate liver fibrosis when compared to alternative diagnostic methods available. It is non-invasive and the rapid turnaround time to obtain results is largely appreciated by patients. The wide availability of FibroScan devices based on Vibration-Controlled Transient Elastography (VCTE) technology, affordability, and its modest requirement to attain technical proficiency required to do the scans mean the method can be rolled out easily across most clinical practices.

FibroScan Versus Liver Biopsy

Currently the gold standard tool to diagnose NAFLD is liver biopsy. However, there are some limitations to this such as invasiveness, complications, the potential for subsequent adverse reactions and relatively high price (Cai et al 2021). Complications of liver biopsy include pain and hypotension which can lead to increased length of hospital stay and cost (Hashemi et al 2016). The mortality rate after percutaneous liver biopsy is one in 10000 to one in 12000 and therefore continuous liver biopsy for follow-up is nearly impossible (Hashemi et al 2016). Hence the application of FibroScan as a non-invasive tool to provide evidence about the progression of NAFLD and can be considered as an alternative diagnostic method to liver biopsy in NAFLD patients.

Literature Review

The role of TE in detecting fibrosis is quite valuable, however it comes with some limitations in overweight patients. As obesity and being overweight are prevalent in people with NAFLD, there is hesitancy on the accuracy of TE in detecting various stages of fibrosis in NAFLD patients. Successful measurement decreases remarkably when it is performed on patients with a body mass index >25kg/m2 (Jiang et al 2018). Therefore, an XL probe was developed to account for this challenge and reduce the failure ratio when using TE technology on obese patients (Jiang et al 2018).

In one study, it was reported that for patients with fibrosis stage of >1, sensitivity was 83.7%, specificity was 78.2%, positive predictive value (PPV) was 92.2%, and negative predictive value (NPV) was 65.6%. In cases with fibrosis stage of ≥2, sensitivity was 87.5%, specificity was 78.4%, PPV was 69.9%, and NPV was 89.5%. When liver fibrosis stage was ≥3, the calculated amounts were 93.7%, 91.1%, 82.4%, and 95.9% for sensitivity, specificity, PPV and NPV, respectively. When fibrosis stage was ≥4 sensitivity reached 96.2%, specificity was 92.2%, PPV 55.5%, and NPV 98.5% (Hashemi et al 2016). It is noteworthy that using TE to diagnose fibrosis has a greater sensitivity and specificity when the pathological fibrosis increases. Study authors concluded that using TE in detecting the level of fibrosis in NAFLD cases has high accuracy and can be a good alternative for liver biopsy for patients who cannot or chose not to undergo invasive procedures.

Although most people with NAFLD do not progress to advanced fibrosis, due to the high prevalence of NAFLD, it is one of the main indications for liver transplantation in Europe and the USA. In fact, it has been reported that 30% of patients with NAFLD may develop non-alcoholic steatohepatitis (NASH), 25% may develop fibrosis, 10-20% may develop cirrhosis, and 4% may develop hepatocellular carcinoma (Jiang et al 2018). Therefore, we can see the benefit in identifying those who are at greatest risk of disease progression and who would benefit from treatment.

Another study combined the FibroScan and aspartate aminotransferase (AST) values (called FAST; FibroScan-AST) to identify patients with varying levels of NAFLD (Newsome et al 2020). They noted that the FAST score provides an efficient way to non-invasively identify patients at risk of progressive NASH and thereby reduce unnecessary liver biopsy in patients unlikely to have significant disease. CAP and LSM by VCTE measurements are widely applicable in patients with NASH, with a low failure rate (3%) and good performance in determining the degree of liver steatosis and fibrosis (Newsome et al 2020).

A meta-analysis was performed on the diagnostic accuracy of TE for staging hepatic fibrosis in patients with NAFLD, and the results were positive. They concluded that TE provides an accurate and feasible imaging technique that enables the staging of hepatic fibrosis in NAFLD (Jiang et al 2018). The authors also went on to note that diagnostic accuracies when using TE are higher in those with advanced liver fibrosis (F3) and cirrhosis (F4), than those with less liver damage (Jiang et al 2018).

An additional study was done to analyze the application of TE for diagnosing steatosis and fibrosis in NAFLD patients. The authors concluded that using CAP and LSM for diagnosing fibrosis was a feasible and accurate method, particularly in those with severe fibrosis and cirrhosis (Cai et al 2021).

Conclusion

The benefits of using TE in a clinical setting cannot be overstated. With liver disease on the rise, non-invasive tools to identify and stage the health of the liver are essential. Routine evaluation of patients with suspected liver disease can assist with proper monitoring and treatment. FibroScan offers clinicians and patients a safe, quick turnaround assessment of liver health.

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Ginger in Women's Health Care: Gynecology and Primary Care

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Ginger in Women's Health Care: Gynecology and Primary Care

Abstract

The well-known herb ginger, or *Zingiber officinale*, has been used for medicinal and culinary purposes for thousands of years throughout the world. Traditionally, ginger has been used to address a variety of ailments including nausea, vomiting, colds, arthritis, and headaches, among others. Although it is not often thought of as an herb for women's health, there are a myriad of clinical uses for ginger to support medical conditions that are women-only as well as some that tend to impact women more often. This article seeks to highlight some of these uses of ginger beyond its antiemetic action by also discussing applications of ginger for dysmenorrhea, heavy menses, migraines, IBS, osteoarthritis, and exercise induced myalgia.

Introduction

Ginger root (*Zingiber officinal Roscoe*) is widely known and used as a spice and seasoning in cuisines and traditional medicine throughout the world. The nonvolatile pungent compounds have well known cardiovascular, gastrointestinal, antioxidant, analgesic, and anti-inflammatory effects (Dugasani et al 2010). Ginger has been shown to reduce pain in osteoarthritis, muscle pains and dysmenorrhea, heavy menstrual bleeding, reduce pregnancy related nausea and vomiting and chemotherapy related nausea, as well as vomiting after surgery. This short article will emphasize the recent research in using ginger for problems of great importance to women including menstrual cramps, heavy menses, nausea/vomiting of pregnancy, migraine pains, muscle pains, nausea of chemotherapy, and osteoarthritis.

Menstrual Cramps

Rather than discuss each of the studies on this topic, we were pleased to see in 2015 a systematic review and meta-analysis of randomized trials on the efficacy of ginger for primary dysmenorrhea (Daily et al 2015). In this analysis, seven randomized controlled trials met the inclusion criteria and these were used for the systematic review. Six of these trials were conducted in Iran and one in India. The meta-analysis of the data from these studies demonstrated a significant effect of ginger in reducing the pain visual analog scale (PVAS), a tool widely used to measure pain, in women having primary dysmenorrhea. In total, these randomized controlled trials showed significant efficacy for primary dysmenorrhea at doses of 750-2000mg per day during the first three to four days of the menstrual cycle.

The cause of menstrual cramps is thought to be due to an increased production of prostaglandins in the endometrium (lining of the uterus). Menstrual blood of women with primary dysmenorrhea has greater amounts of the pro-spasmodic and pro-inflammatory prostaglandins, PGE_2 and PGF_2 alpha. It is thought that the anti-inflammatory properties of ginger are due to the gingerols, which can lead to a reduction in prostaglandins, and inhibit cyclooxygenase-2, NF kappa beta and 5-lipoxygenase (Kiyama 2020).

Heavy Menstrual Bleeding

In this current study, Iranian high school students had regular menstrual cycles and a recent history of at least one heavy menstrual cycle (Kashefi et al 2015). These were girls who also had no gynecological disease, were not regularly taking hormonal medications or NSAIDS, did not have a vaginal or pelvic infection, and were not overweight or obese. Three consecutive menstrual cycles were monitored and scored for blood loss before starting the ginger or placebo. Ginger capsules contained 250mg of dried ginger, and one was given three times daily or placebo capsule three times daily starting from the day before menstrual bleeding until the third day of the menstrual period for a total of four consecutive days for the three months of menstrual cycles.

The level of menstrual blood loss dramatically decreased during the three intervention cycles in the ginger group and was significantly better than in the placebo group. The average decrease in heavy menses in the ginger group started the very first month, was even better the second month, and then a little better the third month. There were no average hemorrhage changes in the placebo group. After the intervention, the ginger group decreased in mean hemorrhage by 46.6% and the placebo group by 2.1%. Three girls had adverse events in each group: ginger=1 heart burn, 1 abdominal pain, 1 diarrhea; placebo=1 abdominal pain, 2 flatulence (Kashefi et al 2015).

Serum levels of prostaglandin E2 and prostacyclin are higher in women with heavy menstrual bleeding, which results in the vasodilatation and local platelet accumulation in addition to lower amounts of prostaglandin F2alpha which is responsible for vasoconstriction. Women with heavy menstrual bleeding also have more PGE2 receptors (Kiyama 2020). It would be logical then that herbs and/or foods and/or medications that inhibit prostaglandin synthesis and leukotriene formation may provide the needed anti-inflammatory effect to decrease heavy menstrual blood loss.

Heavy menstrual bleeding is one of the more common gynecological reasons why women come to their health care provider. It can affect quality of life and cause iron deficiency anemia. Not only can this result in mild to severe fatigue, but changes in cognition, exercise tolerance, dyspnea, and heart palpitations can occur. The bigger picture is determining what is causing the heavy menstrual bleeding, defined as greater than 80ml per menstrual cycle. Causes of heavy menstrual bleeding can include a simple anovulatory cycle due to stress or perimenopause, thyroid disorders, uterine polyps, uterine fibroids, adenomyosis, uterine pre-cancer, uterine cancer, and von Willebrand syndrome. While some common herbs and medicines can be used to treat a particular episode of heavy menstrual flow, treating the underlying condition is particular to each of the causes mentioned.

Migraine Headaches

Migraine headaches are one of the most common causes of pain and can vary from a minimal impact on activities of daily living to incapacitating. An effective herbal intervention for acute pain relief would be a welcome addition to the list of options.

This double-blind randomized controlled clinical trial compared the efficacy of ginger to sumatriptan, a standard conventional prescription treatment, in the treatment of common migraine (Maghbooli et al 2014). Study subjects with common migraines, in Iran, were randomly provided either one ginger capsule of 250mg upon onset of headache or 50mg of sumatriptan. Women comprised 68% of the sumatriptan group vs 74% of the ginger group. Both sumatriptan and ginger powder decreased the mean severity of common migraine attacks within two hours of use. No significant difference existed between the two treatments, which is impressive for the ginger. Before taking the medication, 22% of the sumatriptan group and 20% of the ginger group had severe headaches. The mean headache severity at two hours after sumatriptan or ginger use demonstrated similar effectiveness for both groups. There was 4.7-unit reduction in the headache

severity in the sumatriptan group and a 4.6-unit reduction in the ginger group. Favorable relief was achieved in 70% of the sumatriptan-treated headache individuals and 64% of the ginger-treated patients at two hours following intake. There were more side effects from sumatriptan use including dizziness, sedation, vertigo, and heartburn. The only clinical adverse effect of ginger was dyspepsia.

In a previous study in 2005 using ginger with feverfew in sublingual tablets for acute migraine pain, 32% of participants were pain-free at two hours in those receiving the medication vs 16% receiving placebo. In total, at two hours, 63% receiving medication were either pain-free or had only mild pain vs 39% for placebo (Cady et al 2005).

And in another fever/ginger study for acute migraine treatment in the early pain phase, an open-label study enrolling 30 subjects, male and female, 48% were pain-free after two hours with 34% reporting a headache of only mild severity and 29% having a recurrence within 24 hours (Cady et al 2011).

Ginger and Nausea/Vomiting of Pregnancy

Nausea and vomiting are the most common unpleasant symptoms during pregnancy. Fifty percent to 90% of women experience these complications. In the most recent study on this topic, a single-blind controlled randomized clinical trial was conducted in women up to 20 weeks of pregnancy in Iran (Ozgoli et al 2009). Thirty-two women received ginger and 35 received placebo. One ginger (250mg) or placebo capsule four times per day was given over the course of four days. Nausea intensity improved in 84% of those who used the ginger and in 56% of the women in the control group. The incidence of vomiting in the control group was decreased 9%, while the ginger group experienced a 50% decrease.

At least four previous published studies have shown success in the use of ginger for nausea and vomiting of pregnancy (Chittumma et al 2007, Haji et al 2013, Mohammadbeigi et al 2011, Thomson et al 2014). Doses of 1000mg to 1500mg per day have been used previously. The current study showed not only a positive effect, but women were satisfied with that effect and no complications were observed during the treatment period.

Ginger Extract on Nausea due to Chemotherapy

Nausea can be a significant side effect of numerous chemotherapy medications and preventing and treating chemotherapy induced nausea and vomiting (CINV) is a priority in oncology patients. There are indeed some important and often effective conventional medications, but even then, nausea and vomiting can occur in 30-60% of cancer patients (Rao and Faso 2012). Nausea and vomiting are, at best, unpleasant symptoms, but can significantly affect quality of life, cause insufficient nutrition, and can even result in chemotherapy treatment delays or reduction in desired dosing.

Ginger has been studied for nausea due to other causes such as pregnancy and post-operative nausea and vomiting. There is also a literature review published in 2013 on ginger and chemotherapy induced nausea and vomiting (Marx et al 2013). However, there are some research methodology problems in the previous studies, which might be preventing common use of ginger in these patients in the oncology setting.

The primary objective of this double-blind, randomized placebo-controlled trial (Marx et al 2017) was to address those methodology issues and assess ginger compared to placebo in patients receiving chemotherapy agents that are moderatey to highly associated with causing nausea and vomiting.

Patients were randomly assigned to receive 300mg capsules four times daily of standardized ginger extract or placebo in conjunction with the standard medications for nausea/vomiting for the first three cycles of chemotherapy. Ginger or placebo was given with meals, starting on the day of the chemotherapy and for a total of five days, for each cycle. Over three consecutive chemotherapy cycles, nausea was more prevalent than vomiting. In cycle one, those who received ginger reported significantly better quality of life in terms of chemotherapy induced nausea, nausea/vomiting, as well as less fatigue than placebo. There were no significant results in cycle two. In cycle three, quality of life and fatigue were significantly better in the ginger compared to placebo group (Marx et al 2017).

Ginger and Nausea Associated with Antiretroviral Medication and Postoperative Nausea

Given the evidence showing ginger can reduce nausea related to pregnancy, chemotherapy, and other situations, one might deduce that ginger's antiemetic properties could be extended to other instances when nausea is present. Indeed, one study concluded that a dose of 500mg of *Zingiber officinale* taken by mouth twice daily 30 minutes prior to each dose of antiretroviral medication seems to be effective in treating nausea and vomiting associated with these drugs. Mild, moderate, and severe nausea among study participants was reduced compared with the placebo group ($p=0.01$) (Dabaghzadeh et al 2014). There may also be opportunity for clinical application of ginger to address post-operative nausea. One study found that ginger effectively reduced nausea and vomiting in participants who underwent open nephrectomy as well as laparoscopic nephrectomy. Participants of this study were either given a ginger essence or placebo and severity of nausea was assessed using a visual scale (Hosseini and Adib-Hajbaghery 2015). Another study investigating post-operative use of ginger concluded ginger reduced incidence of nausea and vomiting after various surgical procedures, but these reductions were not statistically significant (Montazeri et al 2013).

Gastrointestinal Motility, Irritable Bowel Syndrome, Inflammatory Bowel Disease

While the antiemetic effect of ginger has been well-established, there is also evidence this herb may be useful in supporting gastric emptying and proper GI motility. A very small study (n=11) sought to determine if ginger had an impact on gastric motility and functional dyspepsia by administering a total of 1200mg of ginger in capsule form one hour prior to having participants eat a 500ml low-nutrient soup. Results showed gastric emptying was faster after ginger compared with placebo, but unfortunately did not result in reduction in gastrointestinal symptoms (Hu et al 2011).

Since ginger contains anti-inflammatory constituents and its antiemetic properties are well documented, there is the notion that ginger may be helpful for IBS. Unfortunately, the evidence on ginger and IBS is underwhelming. One double-blind randomized controlled pilot study on ginger and IBS did not find any statistically significant difference between participants with IBS who received placebo, 1g, or 2g of ginger daily for 28 days (van Tilburg et al 2014). One possible limitation of this study was the small size of 45 total participants.

Research currently underway is aiming to establish if there is a role for ginger in inflammatory bowel disease.

Ginger May Reduce Exercise-induced Muscle Pain and Soreness

This systematic review evaluated the use of ginger as an analgesic and ergogenic aid for exercise-induced pain (Wilson 2015). Other studies have explored ginger as an analgesic, for example with acute migraines and dysmenorrhea. However, this is the first review of ginger as an analgesic and ergogenic aid (an exogenous substance that enhances athletic performance) for exercise training and athletics.

Casual exercisers, serious athletes, and professional athletes all commonly use NSAIDS to prevent and manage pain and as many as 50-70% of athletes take NSAIDS regularly. With known potential side effects, including suppression of muscle protein synthesis, muscle degeneration after exercise, and adverse effects on cartilage repair, NSAIDS are actually counter-productive for active individuals in particular.

PubMed was searched in April 2015 for randomized, controlled trials (RCTs) assessing ginger as an analgesic or ergogenic aid for athletes. Any studies in which ginger was used in combination with other ingredients were excluded. A total of nine publications were identified; seven studies evaluated ginger as an analgesic for athletes, and nine studies evaluated ginger as an ergogenic aid (Wilson 2015).

Studies on the analgesic effects evaluated acute single-dose uses of ginger and longer duration effects of 11 days to six weeks in doses ranging from 2-4g/day. In two RCTs, the acute doses of 2g/day of dried ginger had no significant analgesic effects. The longer duration studies evaluated

different forms of ginger including raw, heat treated, and powdered and were taken before and after exercise. In four RCTs, there were modest benefits (Wilson 2015).

Ginger had no clear benefits in the ergogenic studies. One study found that 4 g/d ginger may accelerate upper body strength recovery after resistance exercise. Daily use of ginger may reduce the inflammatory response to cardiorespiratory exercise (Wilson 2015).

While the studies in this review have some design and reporting flaws and different ginger preparations, we think this before and after exercise use of ginger for reducing muscle pain and soreness has merit. An ideal scenario might be 2 g/day for five to 14 days before an endurance event and then 4g after to accelerate recovery of muscle strength. Acute doses of 2g of ginger appear to provide no analgesic benefit.

Osteoarthritis and Ginger

The evidence demonstrating the efficacy of ginger for osteoarthritis-associated pain is mixed. While some studies did conclude oral ginger reduced pain in participants with osteoarthritis, other trials found ginger to be an ineffective means of pain management. This is especially true of topical ginger preparation. One meta-analysis found taking ginger orally at a dose of 500 to 1000mg daily with trial duration ranging from 3 to 12 weeks resulted in positive outcomes including statistically significant pain reduction in ginger groups compared with placebo. Adverse events were characterized as mild and limited to complaints of dyspepsia and “bad taste” (Bartels et al 2015). On the contrary, a different meta-analysis concluded approximately 500 to 1000mg of ginger taken daily for 6 to 12 weeks did not yield sufficient evidence to support the use of ginger to reduce pain or improve function (Araya-Quintanilla et al 2020). Despite some conflicting data on ginger as a standalone therapy for osteoarthritis, a recent study investigating treatment of mild osteoarthritis found a combination product called Tregocel®, which contains curcuminoid extracts of herbs including *Harpagophytum procumbens, Boswellia serrata, Apium graveolens*, and *Zingiber officinale*, to be efficacious for pain reduction compared with standard osteoarthritis therapy (Zegota et al 2021).

Cautions, Contraindications, and Adverse Reactions

As with all supplements and botanical medicines, it is important to discuss initiation of anything new with a doctor or other licensed health care provider. Although supplements are often viewed by the public as relatively innocuous, there are many potential interactions between herbs, pharmaceuticals, and nutrients to be aware of. Caution is advised in the combination of ginger with anticoagulant and antiplatelet drugs since ginger may slow clotting and thus increase chances of bleeding or bruising with certain medications (Abebe 2002, Marx et al 2015). There are some animal studies showing theoretical interactions between ginger and calcium channel blockers, cyclosporine, losartan, metronidazole, and antidiabetic drugs. Overall, ginger is typically well-tolerated. However, especially when taken at higher doses, some patients endorse

GI symptoms including dyspepsia, diarrhea, heartburn, belching, and unpleasant taste. For topical applications, burning sensation or contact dermatitis may occur.

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Vitamin D: Focus on Immune Modulation

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Vitamin D: Focus on Immune Modulation

Abstract

Vitamin D supplementation is not strictly for healthy bone metabolism. There is a significant expression of vitamin D receptors (VDRs) in specific target cells and tissues. Vitamin D deficiency is very common around the world, being affected by latitude/winter season, melanin production, pharmaceutical side effects, obesity, and fat malabsorption disorders. Deficient serum vitamin D levels modulate VDR expression which influences expression of downstream genes and induces protein cascades in different tissues to elicit disease symptoms. The common theme in all of the studies reviewed is the role vitamin D plays in the immune response. Vitamin D deficiency has been implicated in not only immune related conditions, but chronic medical conditions as well. While the mechanism of action of vitamin D in these conditions has not been fully elucidated, significant associations have been documented. This suggests vitamin D may provide effective, non-invasive, and non-pharmaceutical interventions for treatment and prevention of many diseases. The present review will summarize key findings from various literature reviews and meta-analyses of *in vitro* studies, *in vivo* studies, as well as human clinical trials, to provide evidence for the role of vitamin D in immune modulation.

Introduction

Vitamin D has a well established role in calcium and phosphorus homeostasis, vital to optimal mineralization of bones and teeth. It is not surprising that individuals with a vitamin D deficiency present with conditions such as osteomalacia, osteoporosis, and periodontitis. However, due to vitamin D's various target tissues and cells, a deficiency can cause patients to present with symptoms that are not related to bone health.

In order to understand the physiological activity of vitamin D, identification of its receptor in specific cells and tissues is crucial. Vitamin D receptor (VDR) are abundant in small intestinal epithelium, large intestine, pancreatic beta islet cells, distal renal tubular epithelial cells, bronchial epithelial cells, epidermal epithelial cells, osteoblasts, T-lymphocytes, monocytes/macrophages, and parathyroid epithelial cells (Wang et al 2012). VDR expression is lower, yet significant, in the center of efferent ducts in testes, prostate gland, and in lobule and ductal epithelial cells in mammary glands (Wang et al 2012). VDR was undetectable in hepatocytes, brain tissue, skeletal, smooth and cardiac muscle, thyroid, and adrenal gland. However, the antibodies used in the immunoassays were not one hundred percent VDR specific (Wang et al 2012). Optimal functioning of these target tissues and cells depends on adequate serum vitamin D levels.

Vitamin D has two main forms: 25-hydroxyvitmain D (25(OH)D), which is produced in the liver, and 1,25-dihydroxyvitamin D (1,25(OH)D), which is the active form of vitamin D, that is produced in the kidneys. In order to determine an individual's vitamin D status, serum 25(OH)D levels are measured (Holick et al 2011). Vitamin D deficiency is defined as a serum level of 25(OH)D below 50nmol/L, while insufficiency is a serum level of 52.5-72.5nmol/L (Holick et al 2011). In order to consistently raise serum levels above 75nmol/L, at least 1000IU per day of vitamin D is required in all age groups; however, various dosages above 1000IU are required to correct the deficiency depending on age, pregnancy, and use of certain medications (anticonvulsants, glucocorticoids, and antifungals) (Charoenngam and Holick 2020, Holick et al 2011). The main cause for deficiency is the lack of exposure to sunlight due to sunscreen use, dark skin pigmentation, and the winter season (Charoenngam and Holick 2020). Other significant factors that are associated with vitamin D deficiency are body mass index greater than 30, fat malabsorption disorders (such as celiac disease, bile insufficiency, irritable bowel disease, and cystic fibrosis), liver and kidney failure, medications such as anticonvulsants, drugs used to treat HIV/AIDS, corticosteroids, rifampicin, and primary hyperparathyroidism (Charoenngam and Holick 2020, Holick et al 2011). One of the main target cells that are greatly affected by inadequate serum vitamin D levels are those of the immune system.

Vitamin D and the Immune System

Over the last decade the immunological role of vitamin D has become more evident. More recently, a deficiency of vitamin D has been associated with immune related conditions and diseases such as cancer, viral respiratory infections, and SARS-CoV-2 infection (Carlberg and

Velleuer 2021, Charoenngam and Holick 2020, Davari et al 2021 Ghasemian et al 2021, Herr et al 2011). Vitamin D deficiency has also been implicated in many chronic medical conditions such as uterine fibroids, metabolic syndrome, cardiovascular disease, and polycystic ovarian syndrome (PCOS), all of which have some inflammatory component to disease progression (Cai et al 2021, Ciebiera et al 2018, Gokosmanoglu et al 2020, Theik et al 2021). Awareness of the role that Vitamin D has in both the innate and adaptive immune responses can help to understand how and why vitamin D deficiency is associated with the aforementioned conditions.

The cells of the innate and adaptive immune system have the ability to convert 25(OH)D to its active form, 1,25(OH)D. They also express VDR, which is a nuclear receptor that can influence gene expression, so that the 1,25(OH)D can induce antimicrobial responses in those cells (Baeke et al 2010). During an infection, 1,25(OH)D is produced within monocytes and macrophages, which stimulates antimicrobial activities of these immune cells through an autocrine signaling cascade initiated by VDR binding and gene expression of cytokines, chemokines, pattern recognition receptors, and antimicrobial peptides (Baeke et al 2010, Biriken et al 2021, Charoengam and Holick 2020). It also influences the immune response of neighbouring lymphocytes by upregulating T_H2 and Treg cells and downregulating B, T_H1, and T_H17 cells, effectively suppressing the proinflammatory state (Cantorna et al 2015, Charoengam and Holick 2020, Holick 2007). Vitamin D downregulates B lymphocyte antibody production (Charoengam and Holick 2020). The lack of vitamin D in target cells and tissues can help to explain the presence of disease symptoms and why supplementation may be used as an adjunct therapy for symptom relief and suppression of disease progression. Vitamin D and its effects can be clearly seen when studying viral infections.

Viral Respiratory Infections: Special Focus on COVID-19

The course of a respiratory tract infection depends on the innate and adaptive immune response. Since vitamin D has demonstrated a strong influence on immune cell function (Charoengam and Holick 2020), it is probable that vitamin D levels can affect incidence and severity of a viral infection. At optimal levels, vitamin D causes enhancement of the lung epithelial cell barrier, stimulates maturation of type 2 pneumocytes, promotes surfactant production, and increases the innate immune response within the airways (Costagliola et al 2021B). Recent studies have shown that vitamin D deficient status is associated with increased incidence and severity of viral respiratory infections (Lai et al 2017, Martineau et al 2017). This association is stronger in patients with lung disease, such as asthma, COPD (Ginde et al 2009) and COVID-19 (Kazemi et al 2021).

A key theme that has emerged from observing and treating patients with SARS-COV-2 infection is immune hyperinflammation, making immunomodulation a possible treatment strategy (Tan et al 2020). The cytokine storm that is created during this infection leads to acute respiratory syndrome, organ failure and, in many cases, death (Musavi et al 2020). In a systematic review and meta-analysis of 15 recent studies, Kazemi et al revealed that there is an association between

vitamin D deficient status and severity of COVID-19 disease (Kazemi et al 2021). In other words, patients who are vitamin D deficient suffer from a more severe SARS-COV-2 infection (Alsafar et al 2021, Lau et al 2020). The greater the cytokine storm, the more severe the infection is (Alsafar et al 2021, Mustavi et al 2020). The role of vitamin D as a therapeutic agent comes into play here by inducing an anti-inflammatory response and suppressing the production of proinflammatory cytokines (Charoengam and Holick 2020). A recent RCT studying patients with mild to moderate COVD-19 symptoms demonstrated that supplementing patients with 5000IU of vitamin D orally per day for two weeks significantly reduced the symptoms of cough and ageusia (Sabico et al 2021).

It has been posited that, in an effort to control SARS-COV-2 viral replication, vitamin D induces numerous antimicrobial pathways, which reduce serum vitamin D levels quicker than the body can replenish them back to sufficiency. The antimicrobial response is then muted once vitamin D insufficiency is present (Lau et al 2020). However, this effect does not last as the body is capable of recovering from the acute inflammatory response allowing vitamin D levels to rise again (Smolders et al 2021). Unfortunately, this reaction was demonstrated in only nine healthy male volunteers. While these volunteers had insufficient vitamin D levels, patients with severe COVID-19 disease had marked vitamin D deficiency (Karonova et al 2021), which could make recovery from the acute inflammatory response more difficult.

Current investigations have demonstrated the role of vitamin D in inducing an antiviral response and negatively regulating the renin-angiotensin-aldosterone system (RAS). (Costagliola et al 2021A). The RAS consists of two protein axes, which are ACE/Ang II/ATR and ACE2/Ang 1-7/MasR (Musavi et al 2020). SARS-COV-2 infection disrupts this balance and causes lung damage, whereas vitamin D upregulates ACE2 receptor expression providing a protective effect on lung tissue (Musavi et al 2020). It is also through this mechanism that vitamin D protects against hypertension and inflammation by inhibiting RAS activity and suppressing renin synthesis (Musavi et al 2020). While much remains unknown regarding SARS-COV-2 infection and more large-scale studies are required, vitamin D may be a possible adjunct treatment and prevention strategy for severe COVID-19 disease.

Anti-Tumor Activity

Vitamin D not only affects the immune system in response to a microbial infection, but also its response to malignant cells. The human body is capable of searching for and destroying cancerous cells. Malignant tumor cell survival relies on genes and immune pathways, some of which are regulated by vitamin D (Carlberg and Velleuer 2021). In addition, *in vitro* studies using human colon cancer cell lines have shown that vitamin D has direct effects on differentiation, proliferation, and apoptosis of neoplastic cells by altering specific gene expression (Carlberg and Velleuer 2021, Palmer et al 2003, Wood et al 2004). Vitamin D also has indirect effects on tumor cell survival by regulating immune cells (Carlberg and Velleuer 2021). Vitamin D induces autophagy of cancerous cells by genomic and non-genomic pathways

to regulate cell proliferation and differentiation (Bhutia 2021). Animal models as well as human and cancer cell lines have shown that vitamin D has antiproliferative effects by influencing specific genes involved in cancer cell growth (Banerjee and Chatterjee 2003).

Many *in vitro* studies have shown that vitamin D demonstrated anti-tumor activity when it was used to treat several cancer cells lines. Recent work confirms the presence of VDR in glioma cells since stimulating glioma cells lines with vitamin D increased VDR expression and subsequent anti-tumor effects (Lo et al 2021). Cell cycle arrest is the most well documented mechanism of how vitamin D exerts its anti-cancer effects in numerous glioblastoma cell lines (Lo et al 2021). Vitamin D works synergistically with temozolomide to significantly increase apoptosis in the C6 rat glioblastoma cell line (Bak et al 2016). Treating breast cancer cells with vitamin D and its analog, EB1089, also induces apoptosis through a VDR-mediated signaling cascade that suppresses the anti-apoptotic protein Beclin-2, which is normally overexpressed in tumors (Hoyer-Hansen et al 2005). Downregulation of Beclin-2 was also demonstrated in prostate cancer cell lines (Guzey et al 2002). Vitamin D and its analogs have also been shown to have anti-invasive effects in glioma cell lines (Lo et al 2021). An interesting new find is that VDR polymorphisms may be a genetic risk factor for several cancers; however, more large-scale association studies are required to confirm this in each individual cancer type (Lo et al 2021). Human trials using vitamin D supplementation in patients with cancer, both invasive and *in situ* types, revealed that supplementation did not prevent cancer, reduce its risk, or affect cancer incidence (Avenell et al 2012, Scragg et al 2018). It is suggested that humans may respond to vitamin D differently leading to the insignificant response in its supplementation (Carlberg and Velleuer 2021). There are numerous factors that affect cancer progression in humans, so if vitamin D is demonstrating anti-proliferative effects *in vitro*, there may still be a therapeutic role for it in treatment strategies.

Uterine Fibroids

Vitamin D has anti-tumor effects not only in cancerous cells, but also in benign tumor cells. One example is uterine fibroids or uterine leiomyomas. The uterine fibroid, derived from the myometrium of the uterus, is the most common benign tumor in women of reproductive age (Vergara et al 2021). Many studies have demonstrated the association of vitamin D deficiency and increased risk of developing uterine fibroids (Baird et al 2013, Li et al 2020, Mitro and Zota 2015, Paffoni et al 2013, Singh et al 2019). *In vitro* studies demonstrated an anti-proliferative effect of vitamin D on human leiomyoma cells, in a concentration and time-dependent manner (Sharan et al 2011). Proteins involved in tumor proliferation, such as cyclin-dependant kinase 1 {CDK-1}, proliferating cell nuclear antigen {PCNA}, catechol-O-methyltransferase {COMT}, and proliferation marker protein KI-67 {MKI-67} were significantly reduced in the presence of vitamin D (Sharan et al 2011). In addition, transforming growth factor beta {TGF-β}, which is responsible for extracellular matrix regulation, was significantly inhibited by vitamin D, leading to decreased fibroid volume (Halder et al 2011). It is well known that uterine fibroids are hormonally regulated. Increased extracellular vitamin D regulates the expression of nuclear

estrogen and progesterone receptors in a dose dependent manner (Al-hendy et al 2015). In recent clinical trials, treating women with uterine fibroids who had a vitamin D deficiency demonstrated tumor growth inhibition (Arjeh et al 2020, Ciavattini et al 2016, Suneja et al 2021) as well as reduction in tumor volume (Hajhashemi et al 2019). It has been suggested that uterine fibroids could be the result, in part, of a chronic pro-inflammatory immune response that is governed predominantly by T_H17 cytokines (Wegienka 2012). Vitamin D can regulate the expression of T_H17 cytokines through activation of its receptor and downstream genes (Charoengam and Holick 2020). This simple treatment may have beneficial implications in female health standards of care for dysmenorrhea and fertility.

Metabolic Syndrome

Chronic infection and systemic inflammation are major contributors to metabolic syndrome (MeS) and insulin resistance. Vitamin D has the ability to modulate the adaptive and innate immune system, hence, it is not surprising that a vitamin D deficiency has been associated with increased incidence of type 2 diabetes, increased risk for MeS, increased triglycerides, decreased high density lipoprotein levels, obesity, and non-alcoholic fatty liver disease (NAFLD) (Barchetta et al 2011, Bea et al 2015, Ceglia et al 2017, Chon et al 2014, Zheng et al 2019).

An inflammatory terrain is associated with the health of the gastrointestinal tract. The small intestine is a major vitamin D targeting tissue, where VDR levels are abundantly expressed (Wang et al 2012, Zeng et al 2020). VDR expression is greater in the distal small intestine, more specifically within Paneth cells, which are also more abundant than in proximal regions (Zeng et al 2020). These intestinal cells are responsible for secreting antimicrobial agents, known as α-defensins, within the lumen of the small intestine modulating bacterial growth. In a narrative literature review of animal and human studies, vitamin D supplementation given to vitamin D-deficient subjects led to an increase in beneficial bacteria, including Ruminococcaceae, *Akkermansia*, *Faecalibacterium*, *Lactococcus*, and *Coprococcus*, and a decrease in *Firmicutes* (Tangestani et al 2021). Therefore, vitamin D signaling is crucial to maintaining the gut microbiome and a deficiency leads to dysbiosis and inflammation (Su et al 2016). Conversely, a sufficient amount of vitamin D can significantly suppress metabolic disorders by improving insulin resistance, reducing plasma triglycerides, and decreasing progression of hepatic steatosis (Su et al 2016). Targeting treatment plans to optimize gut health can have beneficial implications for many conditions characterized by inflammation.

Polycystic Ovarian Syndrome (PCOS)

PCOS is characterized by a polycystic ovarian morphology, hyperandrogenism, and ovulatory impairment, with insulin resistance as the main pathophysiological finding. As with uterine fibroids, there is an association of vitamin D deficiency with the development of PCOS in women of reproductive age (Gokosmanoglu et al 2020). Furthermore, higher androgen levels were associated with vitamin D deficiency in women with PCOS (Gokosmanoglu et al 2020). Supplementing women with vitamin D demonstrated a significant reduction in androgen levels

and significant increase in insulin sensitivity post treatment (Karadag et al 2018). While the pathophysiology of PCOS cannot be fully explained, vitamin D deficiency and its associations with androgen excess and insulin sensitivity sheds light on possible mechanisms. As suggested with uterine fibroids, vitamin D may modulate androgen production through activation of its receptor in ovarian cells.

Conclusion

A key element in all of the conditions and diseases discussed within this review is inflammation. Whether it is acute or chronic, inflammation paves the way for disease progression. Fortunately, vitamin D can influence the inflammatory response in each of the mentioned diagnoses. How do we alleviate vitamin D deficiency? Current standards for treating vitamin D deficiency suggest testing only at-risk individuals. It makes sense that a patient who is likely to develop osteoporosis, based on age, diet, lifestyle and family history, is tested to confirm vitamin D status. However, patients with recurrent infections, cancer, metabolic syndrome, uterine fibroids, or PCOS may not show typical signs of vitamin D deficiency. Overall, recent scientific investigations have demonstrated that vitamin D has a strong impact in treating symptoms and preventing disease progression. It is time to change standards of care. Just as a complete blood count is run as a routine check, perhaps vitamin D levels should be included. Many more clinical trials are necessary to determine a therapeutic vitamin D dose that provides beneficial biological effects in each disease and condition. Having a non-invasive, non- pharmaceutical, and non-surgical treatment strategy can have a positive impact on today's health care system and in people's lives.

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Low-Hyperforin Extracts of St John's Wort are Safe to Combine with Prescription Medications of All Classes and are Effective in Treating Common Mental Health Concerns

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Conflicts of Interest
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Low-Hyperforin Extracts of St John's Wort are Safe to Combine with Prescription Medications of All Classes and are Effective in Treating Common Mental Health Concerns

Abstract

St John's wort *(hypericum perforatum)* (SJW) has both a rich history of use as well as a well-established body of modern human intervention trials showcasing its ability to benefit a wide array of common mental health concerns. However, academic curriculums training healthcare providers of all disciplines highlight the potential for horrifying herb-drug interactions with SJW. It may be the most well-known contraindication across all disciplines of medicine; do not combine SJW with prescriptions of any type! The objective of this review is to showcase evidence forcing a re-evaluation of the long-held notion that SJW cannot be combined with prescriptions. Specific molecules within SJW (hyperforin and hypericin – most relevant being hyperforin) are known inducers of several cytochrome P450 enzyme systems, and thus the interactions so broadly feared. There are now available preparations of SJW that deliver at or below detection limits of these molecules, and an important body of literature clearly demonstrates that these preparations do not induce P450 systems. SJW is an incredibly valuable tool in the front-line of the war that is mental health, and low-hyperforin preparations are a critical tool in the arsenal of integrative healthcare providers working in this realm.

Introduction

St John's wort (*Hypericum perforatum*) (SJW) has had a rich history of use. Herbal texts describe it as sedative, astringent, and pain-relieving. It was also known to have significant antiviral effects which were confirmed in later modern study. Historical indications included excitability, anxiety, depression, and as a nerve tonic. It was also viewed as useful for neuralgia, fibrositis, sciatica, menopausal neurosis, wound healing, and rheumatic pain (Barnes et al 2002, Hoffmann 2003, Mills and Bone 2012).

SJW belongs to the family Hypericaceae. It has nearly global distribution, missing only from polar regions and desserts. It is an aggressive perennial plant with almost exclusively yellow-coloured flowers. It is often considered an invasive weed. When glands on the petals are crushed, a red stain occurs. The modern common name of the plant came from Paracelsus in the 16th century naming the plant "Johannes-blut" ("blut" meaning blood and soil, a term linking German people to their land) and linking the plant to the martyr St John (Wikipedia 2021).

As anyone trained in naturopathic medicine can attest, hyperforin and hypericin are often showcased as the active constituents in St John's wort. All three herbal texts reviewed for this review (Barnes et al 2002, Hoffmann 2003, Mills and Bone 2012), and certainly many others, declare these molecules to be the active constituents of the plant. However, certain critical facts have since emerged.

1. Hyperforin and hypericin are inherently very unstable molecules. While ultra-specialized extraction processes targeting preservation of hyperforin and hypericin contain appreciable amounts of the two molecules, any processing stress to the plant rapidly degrades them (Madabushi et al 2006, Mueller et al 2004, Orth et al 1999).
2. Low hyperforin/hypericin extracts of SJW have been shown to have important magnitudes of efficacy in management of common mental health concerns (Brattstrom 2009, Camfield et al 2013, Friede et al 2001, Schrader 2000, Woelk 2000).
3. Low hyperforin/hypericin extracts of St SJW have been reproducibly demonstrated not to appreciably impact cytochrome P450 enzyme systems, and thus do not impact metabolism of prescription medication (Arold et al 2005, Chrubasik-Hausmann et al 2019, Madabushi et al 2006, Mai et al 2004, Mathijssen et al 2002, Mueller et al 2004, Mueller et al 2009, Will-Shahab et al 2009, Zahner et al 2019).

Stability of Hyperforin/Hypericin

Tremendous research interest exists in achieving stable isolates of hyperforin. It is important to note that no herb-drug interactions with SJW were reported until 1998, very shortly after a new extraction process was developed that preserved significant amounts of hyperforin (Madabushi et al 2006, Mueller et al 2004, Willmar 1997, Willmar 1998). Further advancements in processing methodologies have led to a six-step system that can be found in **Table 1** (Orth et al 1999).

Table 1. Hyperforin-Preserving Processing of SJW

Step	Process
1	Extraction of deep-frozen blossoms (-20C) with hexane.
2	Separation of lipophilic substrates on a silica gel column.
3	Purification with high performance liquid chromatography (HPLC).
4	Evaporation under reduced pressure.
5	Removal of remaining water by freeze-drying.
6	Storage of hyperforin under nitrogen at -20C.

Standard extraction and manufacturing processes lead to very low yields of hyperforin and hypericin (Ang et al 2004, Fuller et al 2018, Gaid et al 2018, Maisenbacher and Kovar 1992). Hyperforin ($C_{35}H_{52}O_4$) makes up 2-4% of crude, dried SJW (Orth et al 1999). A well-studied proprietary extract of SJW (Ze 117) is reported to contain ≤0.2% hyperforin (Brattstrom 2009, Camfield et al 2013, Friede et al 2001, Mueller 2004, Schrader 2000, Will-Shahab et al 2009, Woelk 2000, Zahner et al 2019). Unpublished data from the manufacturer of the low-hyperforin extract prescribed by the Author state that the herbal extract begins with 1.22% hyperforin, yet following the manufacturing process of the final product, hyperforin is nearly undetectable (0.076%). A second unpublished report from yet another company offering low-hyperforin SJW reports the final product as containing below detection limit of hyperforin (<0.1%).

Herb-Drug Interactions with St John's Wort

It is generally accepted that SJW extracts containing less than 1% hyperforin will not interact with prescriptions (Chrubasik-Hausmann et al 2019, Madabushi et al 2006). Chrubasik-Hausmann and colleagues (2019) review 56 unique publications evaluating interaction of SJW with a very wide array of common prescriptions. Hyperforin dose among these studies ranged from 0.04mg to 41mg per day. It is important to note that many of the studies evaluated interaction with multiple prescription medications as opposed to evaluation of one. Only four of the 56 papers evaluated SJW extracts with hyperforin dose of less than 1mg per day. The authors conclude that a high magnitude of safety can be expected from preparations with less than 1% hyperforin, and that >3mg per day of hyperforin would be required to achieve interactions of concern. Others echo the less than 1% hyperforin content as safe, yet recommend a total daily dose of hyperforin to be <1mg (Madabushi et al 2006).

Hyperforin acts as an inducer of several key P450 enzymes (CYP3A4, CYP2C19, CYP2C9) as well as an inducer of p-glycoprotein (ABCB1) (P-pg), a drug efflux pump (Chrubasik-Hausmann et al 2019).

Well-controlled human trials have shown a lack of interaction of low-hyperforin SJW with cyclosporine (Mai et al 2004), low-dose oral contraception (Will-Shahab et al 2009), digoxin (Mueller et al 2004), and irinotecan (Mathijssen et al 2002). A well-validated seven probe drug cocktail showed no impact of low-hyperforin SJW with caffeine, bupropion, flurbiprofen, omeprazole, dextromethorphan, midazolam, and fexofenadine (Zahner et al 2019). Another study found no interaction of low-hyperforin SJW extract with alprazolam, caffeine, tolbutamide, and digoxin (Arold et al 2005). Lack of interaction was again demonstrated with midazolam (Mueller et al 2009).

Soleymani and colleagues (2017) highlight the potential harm from combining hyperforin-rich SJW extracts with medications. They review an alarming number of case reports demonstrating severe and on occasion life-threatening interactions between SJW and a wide array of common prescription medications including immunosuppressants, anticancer agents, cardiovascular drugs, oral contraceptives, and lipid lowering agents.

Hyperforin is certainly a molecule of tremendous research focus. Preclinical evidence is revealing the molecule to be antileukemic (Billard et al 2013), antidepressant (Pochwat et al 2018), neuroprotective (Oliveira et al 2016), apoptotic in cancer cell lines (Hsu et al 2020), inducer of post-stroke neuroangiogenesis (Yao et al 2019), antidiabetic (Novelli et al 2020), protective against acute cerebral ischemic injury (Ma et al 2018), and potentially improving memory and cognition in Alzheimer's (Griffith et al 2010).

Modern extraction processes aiming to preserve the hyperforin content of St John's wort have succeeded, and as reviewed above, there are inevitably important roles for such preparations. However, given the potential for aggressive and dangerous herb-drug interactions with hyperforin-rich SJW extracts, low-hyperforin SJW extracts are a welcome addition to the tool bag of integrative healthcare providers.

Efficacy of Low Hyperforin Extracts of St John's Wort

A selection of human trials of SJW extracts with <1% hyperforin for outcomes of relevance in mental health is presented in **Table 2.** The trials reproducibly demonstrate important magnitudes of benefit. Three of the four studies in **Table 2** compare low-hyperforin SJW with commonly prescribed antidepressants (Friede et al 2001, Schrader 2000, Woelk 2000). Of note, they collectively demonstrate equivalence/superiority of SJW vs medication for treating depression and anxiety, fewer adverse events, and superior tolerability.

Table 2. Human Trials of Low-Hyperforin St John's Wort Extracts in Mental Health

Methods	Outcomes	Reference
RCT 240 participants randomized to SJW extract Ze 117 or fluoxetine. Baseline HAM-D 16-24. Six-week intervention.	HAM-D decreased 11.54 vs 12.2 SJW vs fluoxetine, respectively. CGI and responder rate superior SJW vs fluoxetine. Adverse events 8% vs 23% SJW vs fluoxetine respectively.	Schrader 2000
Multicentre (40 outpatient clinics) RCT 324 participants. Imipramine 150mg/d vs SJW extract Ze 117 500mg/d for six weeks. HAM-D, CGI and PGI as endpoints. Baseline HAM-D 22.4 vs 22.1 SJW vs imipramine.	HAM-D decreased from 22.4 to 12 with SJW, 22.1 to 12.75 with imipramine. CGI and PGI improved equally. SJW significantly superior for tolerability. Four participants assigned to SJW withdrew from trial vs 26 assigned to imipramine.	Woelk 2000
Multicentre 12 month open-label RCT with 440 participants, SJW extract Ze 117 500mg/d. Baseline HAM-D 20.58, CGI 3.99.	HAM-D 12.07 at week 26, 11.18 at week 52. CGI 2.20 at week 26, 2.19 at week 52. 49% of patients reported a total of 504 adverse events, of which 30 (6%) were possibly or probably related to treatment.	Brattstrom 2009
Multicentre RCT 240 participants, baseline HAM-D 16-24, SJW extract Ze 117 500mg/d vs fluoxetine 20mg/d for six weeks.	Significant difference in responder rate 60% SJW vs 40% fluoxetine. Adverse events 25% fluoxetine vs 14% SJW. SJW "Particularly effective in depressive patients suffering from anxiety"	Friede et al 2001

Note

SJW extract Ze 117 contains ≤0.2% hyperforin

Abbreviations

HAM-D = Hamilton Depression Rating Scale

CGI = Clinical Global Impression

PGI = Patients Global Impression

Discussion

Things deemed sacred in medicine can change over time. Clinicians are taught that a great evil is to combine vitamin K with warfarin, yet powerful evidence has since emerged demonstrating vitamin K supplementation as a key strategy for managing warfarin-treated patients with highly variable INR (International Normalized Ratio) (Ford et al 2007, Reese et al 2005, Rombouts et al 2007, Sconce et al 2007).

Low-hyperforin extracts of SJW containing less than 1% hyperforin and delivering a total daily dose of hyperforin of less than 1mg are safe and appropriate to combine with prescription medications of all types. However, hyperforin-rich extracts of SJW are widely available as over-the-counter supplements. Hyperforin-rich extracts of SJW certainly have utility and may achieve outcomes not achievable with low-hyperforin preparations. Using hyperforin-rich extracts requires diligence and caution on behalf of the clinician.

Low-hyperforin SJW extracts are an indispensable tool for the clinician working in the realm of mental health. The author has had the privilege of administering such extracts to over 1000 patients with tremendous success. Offering low-hyperforin SJW extract to patients with principally mental health concerns revolutionized my practice. It is tremendously effective for depression and anxiety. It is the only “natural” treatment ever observed to powerfully impact OCD. It reproducibly stabilizes mania and over time reduces many common presentations of psychosis.

It is incumbent on the clinician to directly verify hyperforin content with the manufacturer of the product they are recommending. The potential harm of combining a hyperforin-rich SJW extract with almost any prescription medication is immense and can be life threatening. Low-hyperforin SJW extracts are safe and effective in treating a wide array of common mental health concerns.

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Polymorphisms in *IRS1* and *FABP1* Modify Insulin Resistance in Response to Fatty Acid Composition in Non-Diabetic Subjects With Abdominal Obesity: A Clinical Randomized Trial

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Running Head: Genetics and fat quality modify insulin resistance.

Abbreviations: ADRB2, adrenoceptor Beta-2; ADIPOQ, adiponectin; FA, fatty acid; FABP1, liver fatty acid binding protein; FTO, fat mass and obesity-associated gene; HOMA-β, Homeostasis model assessment of beta-cell function; HOCO, high-oleic acid canola oil; HOMA-IR, homeostasis model assessment of insulin resistance; IR, insulin resistance; IRS1, Insulin receptor substrate-1; MCFA, medium-chain fatty acid; MUFA, monounsaturated fatty acid; PLIN, perilipin-1; PPARγ, peroxisome proliferator-activated receptor gamma; PUFA, polyunsaturated fatty acid; RCO, regular canola oil; SFA, saturated fatty acid; SNP, single nucleotide polymorphism; TCF7L2, transcription factor 7-like 2.

Clinical Trial Registry: The trial was registered at clinicaltrials.gov as NCT02029833.

Polymorphisms in *IRS1* and *FABP1* Modify Insulin Resistance in Response to Fatty Acid Composition in Non-Diabetic Subjects With Abdominal Obesity: A Clinical Randomized Trial

Abstract

Background: The prevalence rates of insulin resistance (IR) and its health consequences are increasing worldwide. Emerging evidence suggests a modulatory effect of single nucleotide polymorphisms (SNPs) on IR response to dietary fatty acid (FA) composition; yet, evidence from clinical trials is missing.

Objective: We evaluated the response of IR measures to different levels of dietary FA composition, and the impact of IR-associated genetic polymorphisms on this response.

Methods: Non-diabetic adults (n=116) with abdominal obesity were included in a randomized, controlled-feeding, double-blinded, crossover, multicentre trial. During each phase, participants consumed one of three treatment oils (20% of total fat) for six weeks, separated by a four to 12 week washout. Treatment oils included two-high monounsaturated FA (MUFA) oils, conventional canola or high-oleic acid canola, or a low-MUFA high-saturated FA (SFA) oil blend. Genotyping of nine candidate SNPs was performed using qualitative PCR System.

Results: In the sample as a whole, no differences across the three diets were observed for fasting concentrations of glucose, insulin, and fructosamine, as well as on HOMA-IR or HOMA-β. In the homozygotes of *FABP1* rs2241883-CC, but not in other genotypes, reductions in HOMA-IR (p=0.042) and fasting insulin (p=0.06) were observed following consumption of the high-SFA diet compared to the high-MUFA diets. Reductions in insulin (p=0.04), HOMA-β (p=0.02) and HOMA-IR (trend, p=0.07) in the *IRS1* rs7578326-GG homozygotes were also observed upon consumption of the high-SFA diet compared to the high-MUFA diets. Polymorphisms within *ADIPOQ, ADRB2, FTO, PLIN1, PPARγ*, and *TCF7L2 genes* failed to modulate the effect of MUFA consumption on glucose homeostasis and IR measures.

Conclusion: Dietary FA composition modified IR measures in the carriers of either the *FABP1* rs2241883-CC or *IRS1* rs7578326-GG genotype. These results may contribute to developing an effective genotype-based dietary recommendation to reduce IR incidence and associated complications.

Key words: monounsaturated fatty acid, fatty acid composition, genotype, gene-nutrient interaction, IRS1, FABP1, glucose, insulin resistance, abdominal obesity.

Introduction

Insulin resistance (IR) increases the risk of type 2 diabetes and several metabolic abnormalities (Qi et al 2011, Riccardi et al 2004). Growing evidence underscores the role of dietary fatty acid (FA) composition in the development of IR (Vessby et al 2001). The quality of dietary fat potentially impacts the efficiency of action of insulin signaling pathways by modifying cellular membrane fluidity and/or increasing intramuscular lipid content (Galgani et al 2008, Lovejoy 2002). Also, dietary fat quality may be involved in IR risk by influencing glucose-stimulated insulin secretion (Vessby et al 2001). Dietary FAs with greater degrees of saturation and/or chain length were suggested to negatively affect the secretion and sensitivity of insulin (Lovejoy 2002, Turner et al 2009). In contrast, monounsaturated FA (MUFA) consumption has been reported to ameliorate IR compared to a saturated FA (SFA)-rich diet in non-diabetic subjects (Vessby et al 2001, Due et al 2008, Errazuriz et al 2017) and carbohydrate-rich diet in diabetic patients (Paniagua et al 2007). Yet, the evidence is inconsistent (Haghighatdoost et al 2012, Xiao et al 2006, Lovejoy et al 2002, Chang et al 2016). Genetic predisposition may contribute to the responsiveness of IR to dietary FA, and therefore, may explain the discrepancies in the available evidence.

Many polymorphisms have been identified to be associated with IR risk, independent of insulin secretion, with an estimated heritability of 60% in familial and twin studies (Fall and Ingelsson 2014, Brown and Walker 2016, Mansego et al 2012). Among the IR-associated genetic variants, those within the insulin receptor substrate-1 (*IRS1*) gene have garnered more attention. *IRS1*, encoding a major signaling adaptor protein for insulin, is expressed in insulin-sensitive tissues and plays a pivotal role in insulin-stimulated signaling pathways (Li et al 2016, Alharbi et al 2014). Reductions in the activity and/or expression of IRS1 could contribute to IR and type 2 diabetes (Sesti et al 2001, Zheng et al 2013, Cheng et al 2017). Another candidate gene for IR is FABP1 which encodes the liver fatty acid binding protein and serves as a key regulator of lipid metabolism (McIntosh et al 2014). Polymorphisms that alter the functionality of FABP1 may modify hepatic triglyceride accumulation and FA flux to the liver and, thus, may influence hepatic IR (Mansego et al 2012, Newberry et al 2006). As such, emerging evidence suggests a role of genetics in explaining the action of FA composition in modulating IR (Corella et al 2006, Zheng et al 2015).

Given the increasing prevalence of IR and its metabolic and health consequences worldwide, establishing effective personalized prevention and treatment strategies for IR and its associated metabolic abnormalities requires investigation of the interactions between dietary factors and common genetic variants. Therefore, we hypothesized that polymorphisms within IR-related genes would modulate insulin sensitivity responses to dietary FA composition in subjects with abdominal obesity.

Methods

Study Design and Population

This study was part of the Canola Oil Multi-center Intervention Trial II (COMIT II); a randomized, controlled, double-blinded, crossover study aimed to evaluate the effects of MUFA consumption on body composition and cardiovascular disease-related metabolic responses in individuals with abdominal obesity. Recruitment was conducted from 2014-2016 at four centers including: the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN) at the University of Manitoba in Winnipeg, the Canadian Centre for Agri-Food Research in Health and Medicine (CCARM) at St. Boniface Hospital Albrechtsen Research Centre in Winnipeg, the Institute of Nutrition and Functional Foods (INAF) at Laval University in Québec City, as well as the Department of Nutritional Sciences, The Pennsylvania State University in University Park. Additionally, St. Michael's Hospital in Toronto participated in sample analyses.

Participants aged 20-65 years were included in the trial if they had abdominal obesity as identified with waist circumference of greater than 94cm for men and 80cm for women, in addition to at least one of the following metabolic syndrome parameters as secondary inclusion criteria: fasting blood glucose of ≥5.6mmol/L (according to the American Diabetes Association definition for pre-diabetes), TG ≥1.7mmol/L, HDL-C <1mmol/L (men) or <1.3mmol/L (women), and blood pressure ≥130mmHg (systolic) and/or ≥85mmHg (diastolic). Individuals were excluded if they had kidney, diabetes, liver, or unstable thyroid disease. Current smokers, pregnant and lactating women, individuals consuming more than two alcoholic beverages per day, individuals taking medication known to affect lipid metabolism for at least the last three months, or individuals who were unwilling to stop taking any supplements for at least two weeks before the study were not eligible to participate. Participants were randomly assigned to one of six treatment sequences using a random number generator at randomization.com.

Statement of Ethics

Written informed consent was obtained from all participants prior to enrollment. The protocol was reviewed and approved by institutional ethics boards of the participating clinical sites. The trial was registered at clinicaltrials.gov as NCT02029833.

Dietary Intervention

This study consisted of three six-week treatment periods separated by washout periods of four to 12 weeks. During each treatment phase, participants consumed a controlled iso-caloric, full-feeding diet containing 35% fat, 50% carbohydrate, and 15% protein of total energy, as well as ~208mg/3000kcal/d cholesterol and ~38g/3000kcal/d fiber. All three phases were identical except for the type of treatment oil provided. All meals were prepared based on a seven-day rotating menu cycle in the metabolic kitchen of the participating sites. During the washout periods, participants were instructed to consume their habitual diets. To eliminate the effect of physical activity on IR, participants were requested to maintain their usual level of physical activity during the study.

Treatment oils consisted of 20% of total energy and were incorporated into two equal portions of smoothie beverages consumed at breakfast and supper. Treatment oils included: 1) regular canola oil (RCO; Canola Harvest Canola Oil, Richardson International, MB, Canada) consisted of 6.6% SFA, 65.3% MUFA, 19.6% n-6 PUFA, 8.5% α-linolenic acid, 2) high-oleic acid canola oil (HOCO; Canola Harvest Canola Oil, Richardson International, MB, Canada) consisted of 6.7% SFA, 75.9% MUFA, 14.8% n-6 PUFA, 2.6% α-linolenic acid, and 3) a high-SFA low-MUFA control oil consisted of 22.1% long-chain SFA, 18.1% medium-chain FA (MCFA), 22.0% MUFA, 29.6% n-6 PUFA, 8.2% n-3 PUFA α-linolenic acid. The high-SFA oil blend was prepared using 34.9% safflower oil (eSutras, Illinois, USA), 36.0% ghee/butter oil (Verka, New Delhi, India), 16.0% coconut oil (eSutras, Illinois, USA), and 13.1% flaxseed oil (Shape Foods, MB, Canada). Compliance was assessed by the consumption of smoothies; 90% was the required target for smoothie consumption at each phase. To ensure optimal compliance, participants were required to consume one smoothie each day under the supervision of a clinical coordinator.

Biochemical Measurements

On the first two and last two days of each treatment phase, 12-hr fasting blood samples were collected, processed, and stored at −80°C until further analyzed. Frozen samples were shipped to St. Michael's Hospital (Toronto, ON, Canada) for analysis. Serum insulin and fructosamine were measured with the Roche/Hitachi cobas® immunoassay analyzer and electrochemiluminescence immunoassay kits (Roche Diagnostics, Laval, QC, Canada). Serum glucose was determined using cobas® enzymatic reagents on Roche/Hitachi c501e automated clinical chemistry analyzers (Roche Diagnostics, Laval, QC, Canada). The average of the last two days was calculated and used for endpoint-to-endpoint comparison in statistical analyses.

Homeostasis model assessment (HOMA)-IR and HOMA-beta cell function (β) indices were calculated using the average of the last two days via following formulas (Song et al 2007):

$$\mathrm{HOMA-IR} = \frac{\text{Fasting insulin } \left(\frac{\mu\mathrm{IU}}{\mathrm{ml}}\right) \times \text{Fasting glucose } \left(\frac{\mathrm{mg}}{\mathrm{dl}}\right)}{22.5}$$

$$\mathrm{HOMA-\beta} = \frac{\text{Fasting insulin } \left(\frac{\mu\mathrm{IU}}{\mathrm{ml}}\right) \times 20}{\text{Fasting glucose (mg/dl)}} - 3.5$$

Genotyping

Buffy coat samples of the first day of the first phase were used to extract the genomic DNA using Qiagen DNeasy Blood and Tissue Kit according to the manufacturer's instructions (Qiagen Sciences Inc., Toronto, ON, Canada). The concentration and purity of the extracted DNA were assessed using Thermo Scientific NanoDrop 2000 micro-volume spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). TaqMan GTXpree Master Mix with allele-specific

probes (Applied Biosystems, Life Technologies Inc., Burlington, ON, Canada) was used to genotype nine candidate single-nucleotide polymorphisms (SNPs) within eight genes that have been previously associated with insulin resistance. The selected genes included adiponectin (*ADIPOQ*); adrenoceptor beta-2 (*ADRB2*); fat mass and obesity-associated gene (*FTO*); insulin receptor substrate-1 (*IRS1*); liver fatty acid binding protein (*FABP1*); perilipin-1 (*PLIN1*); peroxisome proliferator-activated receptor gamma (*PPARγ*); transcription factor 7-like-2 (*TCF7L2*). The characteristics of the selected SNPs are presented in **Table 1**. Amplification and detection of DNA were conducted with the 7500 Fast Real-Time PCR System (Applied Biosystems, Life Technologies Inc, Burlington, ON, Canada) and StepOne 2.1 (Applied Biosystems, Life Technologies Inc., Burlington, ON, Canada). All samples were run in duplicate.

Statistical Analyses

The COMIT II sample size was calculated to detect an effect in android fat mass. Statistical analyses were performed using SAS 9.4 (SAS, Cary, NC) based on a per protocol approach. Normality was assessed using the Shapiro–Wilk test and the skewness value. Non-normally distributed variables were log-transformed before analysis. The results are expressed as least square means ± SEMs unless otherwise specified and statistical significance was set at P-value < 0.05. PROC MIXED with repeated-measure procedure was used to assess the effect of the three dietary treatments on IR and glycemic homeostasis. Treatment, sex, age, and genotype were used as fixed effects, participants as a repeated factor. Random effects were identified as treatment sequence, clinical site, and participant. Pre-specified potential confounders such as ethnicity, baseline body composition, baseline fasting glucose level, and HOMA-IR were investigated in all models. The effects of possible gene-MUFA interactions on IR and glycemic homeostasis were assessed using the same approach. However, due to the considerably comparable MUFA concentrations in the two canola treatments compared to the high-SFA treatment, the statistical analysis of the interaction between diet and genetic polymorphism was conducted to compare the combined effect of the two MUFA diets versus the high-SFA diet. In addition to the analysis of the effect of each SNP separately, possible effects of their interaction on IR and glycemic homeostasis were also evaluated using the same approach. The Hardy-Weinberg equilibrium was assessed with χ^2 test.

Results

A total of 125 participants completed the trial. Three participants were excluded due to high fasting blood glucose levels (>7.0mmol/L), and six participants were excluded due to large body weight changes (>5% endpoint-baseline weight change), therefore, 116 participants (72 women and 44 men) were included in this study of the effect of FA composition on IR and glycemic homeostasis. All participants were non-diabetic and mean HOMA-IR was above 3.0 at baseline (data not shown) as well as at endpoint of dietary interventions (**Table 2**). No differences were observed in fasting concentrations of glucose, insulin, or fructosamine, nor HOMA-IR or

HOMA-β indices following the consumption of HOCO or RCO compared to the high-SFA treatment (Table 2).

Only 101 participants (60 women and 41 men) provided a consent for genetic analyses. The ethnicity for the majority (73%) of participants was Caucasian. Participant characteristics are presented in **Table 3**. Genotype-frequencies did not differ from Hardy–Weinberg equilibrium except for the *ADIPOQ* rs266729. The genotype associations with glycemic response to different dietary MUFA levels (**Table 4**) showed that consumption of the high-SFA diet induced reductions, compared to the combined high-MUFA diets, in insulin (high-SFA: 11.8 ± 3.6 and high-MUFA: 13.6 ± 3.5, p=0.04), HOMA-β (high-SFA: 151 ± 46 and high-MUFA: 178 ± 43, p=0.026), and a tendency in HOMA-IR (high-SFA: 2.6 ± 0.9 and high-MUFA: 3.1 ± 0.9, p=0.07) only in the *IRS1* rs7578326-GG homozygotes. No effects of different dietary MUFA levels on IR or glycemic homeostasis were observed in the carriers of the *IRS1* rs7578326-A allele. The *IRS1* rs2943641 polymorphism did not modify IR or glycemic responsiveness to MUFA modification.

The combination of SNPs rs2943641 and rs7578326 within *IRS1* was interrogated and yielded three genotype combinations; rs2943641-C + rs7578326-A (n=92), rs2943641-TT + rs7578326-A (n=2), and rs2943641-TT + rs7578326-GG (n=7). The *IRS1* rs2943641-TT + rs7578326-A combination was excluded from the final statistical analysis due to low frequency. The combination of rs2943641-TT and rs7578326-GG genotypes reduced insulin high-SFA: 11.8 ± 3.7 and high-MUFA: 13.7 ± 3.6, p=0.034), HOMA-β (high-SFA: 150.7 ± 46.3 and high-MUFA: 178.3 ± 43.6, p=0.023), and HOMA-IR (high-SFA: 2.6 ± 1.0 and high-MUFA: 3.1 ± 0.9, p=0.06) levels in response to the high-SFA diet compared to higher MUFA consumption (**Figure 1**). Measures of IR and glycemic control did not respond to the dietary intervention in the carriers of the combination of the *IRS1* rs2943641-C + rs7578326-A.

The results also revealed a reduction (high-SFA: 4.3 ± 0.9 and high-MUFA: 4.5 ± 0.8, p=0.042) in HOMA-IR and a concomitant reduction (high-SFA: 16.7 ± 3.3 and high-MUFA: 17.9 ± 3.2, p=0.06) in fasting insulin concentrations following consumption of the high-SFA diet compared to the high-MUFA diets in the *FABP1* rs2241883-CC homozygotes. No significant FA-by-gene interactions were observed in the other six candidate SNPs within *ADIPOQ*, *ADRB2*, *FTO*, *PLIN1*, *PPARγ*, and *TCF7L2* on the responses of fasting insulin, fasting glucose, HOMA-IR, and HOMA-β to dietary FA modification. Furthermore, none of the nine selected polymorphisms modulated fructosamine levels in response to dietary MUFA (data not shown).

Discussion

Genetic architecture may modulate insulin sensitivity responses to dietary FA composition. This study revealed beneficial effects of a low-MUFA high-SFA fat consumption pattern on IR measures in the *IRS1* rs7578326-GG homozygotes and, for the first time, to our knowledge, in the carriers of the *FABP1* rs2241883-CC genotypes compared to the high-MUFA diets. These

results may, partly, explain the inter-individual variability in the responsiveness of IR to dietary FA modifications.

The current report failed to find overall favorable effects of high-MUFA consumption on IR measures in these non-diabetic subjects with abdominal obesity. Consumption of MUFA-rich diets (>20% of total energy) for three months (Vessby et al 2001) and six months (Due et al 2008) has been previously reported to ameliorate HOMA-IR compared to SFA-rich diet (>15% of total energy). However, studies with shorter durations showed contradictory results regarding the effects of MUFA on glucose homeostasis and IR (Xiao et al 2006, Lovejoy et al 2002). Therefore, the six-week duration of the current study might have been insufficient to permit identification of the proposed beneficial effect of MUFA on IR and glucose homeostasis in our participants with abdominal obesity. Further, genetic predisposition and, possibly, interactions with dietary FAs may modify the individual response to dietary intervention. For instance, even though SFA consumption has been repeatedly reported to be positively associated with IR and hyperinsulinemia (Riccardi et al 2004, Vessby et al 2001, Due et al 2008), Luan et al. showed a genetically-oriented response of fasting insulin concentrations to dietary SFA intake mediated by the *PPARγ* rs1801282 polymorphism (Luan et al 2001). Similarly, the present study results shed light on genetically-driven responses of IR measures to dietary FA composition.

The rs2943641 and rs7578326 polymorphisms within *IRS1* have been reported to modulate IR in different ethnic populations (Qi et al 2011, Li et al 2016, Alharbi et al 2014, Rosta et al 2017). The *IRS1* rs2943641 is the best-associated variant with type 2 diabetes and IR in *IRS1* locus (Qi et al 2011, Yiannakouris et al 2012), and has been marked as a potential functional variant (Rung et al 2009). While the function of the *IRS1* rs7578326 is not defined yet, the A-allele was found to be associated with a higher risk for type 2 diabetes (Zheng et al 2013, Yoshiuchi 2013). The latter polymorphism is located in the intron region which might impact splicing and hence the expression of the functional isoform of the IRS1 protein. The upregulation of *IRS1* expression might alleviate IR and restore the impairment of insulin signaling pathways (Cheng et al 2017).

Both IRS1 variants have been reported to modulate the effects of quantity and quality of dietary macronutrients on IR and type 2 diabetes (Qi et al 2011, Zheng et al 2013, Ericson et al 2013). Zheng et al. reported that the rs7578326 and rs2943641 polymorphisms within the IRS1 gene modified IR in response to the SFA-to-carbohydrate ratio and MUFA consumption in two independent populations (Zheng et al 2013). In the present study, we found an improvement in insulin sensitivity, measured by reductions in fasting insulin, HOMA-IR, and HOMA-β, in the IRS1 rs7578326-GG genotype carriers following consumption of the high-SFA low-MUFA diet compared to the high-MUFA diets in a controlled feeding trial. This finding supports the aforementioned study by Zheng et al. which reported that the carriers of the rs7578326-G allele who consumed a low-MUFA diet incurred a significant reduction in HOMA-IR compared to the non-carriers (Zheng et al 2013); however, the reported low-MUFA consumption in their study might reflect high-SFA and/or high-PUFA. A potential mechanism of this interaction might be related to lipid-induced modulatory effect of tyrosine phosphorylation (Samuel et al 2010,

Frangioudakis et al 2005); however, further research is required to reveal the mechanism(s) that coordinate the interaction between dietary FA composition and IRS1 polymorphisms on IR.

The results of this study also indicate that the homozygotes for the combination of rs2943641-TT and rs7578326-GG of *IRS1* (frequency=7%) may have improved insulin sensitivity following high-SFA compared to high-MUFA consumption. The *IRS1* rs7578326 is adjacent and in moderate linkage with the potential functional *IRS1* rs2943641 (r^2=0.79, in HapMap CEU) (Yiannakouris et al 2012). Therefore, the modulatory effect of the combined the rs2943641-TT and rs7578326-GG *IRS1* genotypes on IR might suggest that the rs7578326 regulate insulin signaling through the functional rs2943641. This finding supports the previously reported modulatory effect of the combination of *IRS1* rs7578326-G and rs2943641-T alleles on IR response to dietary intervention (Zheng et al 2013).

The functional *FABP1* rs2241883 (T94A) was found to be associated with type 2 diabetes and IR (16, Xue et al 2016). The physiological role of FABP1, as a key regulator of lipid metabolism, is suggested to be optimized by the presence of threonine at the N-terminal region of the FA binding site (position 94) (Xue et al 2016, Brouillette et al 2004). The rs2241883 mutation (A94/C allele) could reduce the binding capacity of FABP1 to long-chain FA and subsequently, alter normal lipid metabolism (Xue et al 2016). However, the responsiveness of the *FABP1* A94 allele to dietary FA composition has been previously reported (Robitaille et al 2004). In the current study, consumption of the high-SFA diet in the *FABP1* rs2241883-CC homozygotes ameliorated IR compared to non-carriers. Actually, MCFA contributed to ~18% (10.9 g) of the high-SFA treatment of this study compared to a negligible amount in the high-MUFA treatments. The consumption of small amount of MCFAs (≤10-18 g/d) for ≤90 days has been previously reported to induce beneficial physiological effects including reductions in body weight and fat mass (Tsuji et al 2001, Kasai et al 2003), improvement in lipid profile (Kasai et al 2003), as well as amelioration of HOMA-IR (Han et al 2007). Accordingly, in this study, MCFAs could partly counteract the *FABP1* rs2241883-CC genotype (A94A)-associated proposed reduction in the activity of FABP1 thus, inducing beneficial effects on IR. MCFAs, unlike long-chain FAs, are freely permeable into cells and mitochondria, therefore, a reduction in *FABP1* activity may not inhibit their cellular uptake and/or metabolism as well as would reduce FA flux to the liver (Atshaves et al 201, Wein et al 2009). Consequently, we suggest that MCFAs, despite being present in small concentrations, in the high-SFA diet might improve IR by reducing FA flux to the liver in the *FABP1* rs2241883-CC genotype carriers, thereby, probably overriding the effects of other SFA on glucose insulin homeostasis. To the best of our knowledge, this is the first study to evaluate the effect of FA composition-by-*FABP1* rs2241883 interaction on IR, therefore, confirmatory and biochemical studies are required.

In this study, polymorphisms within *ADIPOQ*, *ADRB2*, *FTO*, *PLIN1*, *PPARγ*, and *TCF7L2* genes failed to modulate the effect of MUFA consumption on glucose homeostasis and IR measures. However, conflicting data still exist regarding IR responsiveness to diet-by-gene interactions (Zheng et al 2015, Grau et al 2009, Warodomwichit et al 2009, Perez-Martinez et al

2008). A major strength of the present study is the crossover design with a controlled full-feeding diet and a relatively large number of participants for such a dietary intervention. In the present study, insulin resistance was assessed by HOMA-IR rather than using the gold standard euglycemic glucose clamp technique which might be considered as a limitation. However, HOMA-IR is a feasible and robust tool for the surrogate assessment of IR, especially in non-diabetic subjects, and has been validated across the clamp technique (Sarafidis et al 2007). Although the majority (73%) of study participants was Caucasian, the mixed ethnicity might be considered as another limitation; however, it might, in contrast, provide generalizability of the current findings to the population at large. Another limitation of this study is that we did not control for multiple testing, which may lead to a potential overstatement of our findings.

In conclusion, polymorphisms in *FABP1* and *IRS1* appeared to modulate the IR response to dietary FA composition. The observed interactions between dietary FA composition and genetic variants within *FABP1* and *IRS1* genes on HOMA-IR may reflect a potential clinical benefit on insulin sensitivity following prolonged exposure to dietary FA modifications. These results may eventually contribute to developing an effective genotype-based dietary recommendation to reduce the incidence and associated complications of IR and type 2 diabetes.

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Table 1: Characteristics of the Selected Polymorphisms

Full name of gene	Gene	SNP	Region	Allele	Genotype (N)			MAF%
				Major/minor	MM	Mm	mm	
Adiponectin	*ADIPOQ*	rs266729	Exon/ 5' UTR	C/G	61	30	10	24.8
Adrenoceptor Beta-2	*ADRB2*	rs1042714	Exon	C/G	42	41	18	38.1
Fat mass and obesity-associated gene	*FTO*	rs9939609	Intron	T/A	44	39	18	37.1
Insulin receptor substrate-1	*IRS1*	rs2943641	Intergene	C/T	50	42	9	29.7
		rs7578326	Intron	A/G	49	45	7	29.2
Liver fatty acid binding protein	*FABP1*	rs2241883	Missense	T/C	46	45	10	32.2
Perilipin-1	*PLIN1*	rs894160	Intron	C/T	47	43	11	32.2
Peroxisome proliferator-activated receptor gamma	*PPARγ*	rs1801282	Missense	C/G	78	21	2	12.4
Transcription factor 7-like-2	*TCF7L2*	rs7903146	Intron	C/T	48	46	7	29.7

MAF: minor allele frequency; MM: major allele homozygous; Mm: heterozygous; mm: minor allele homozygous.

Table 2: Fasting Insulin, Glucose, Fructosamine, and Homeostatic Model Assessment of Insulin Sensitivity and Β-Cell Function According to Dietary Intervention[1]

Variable	Total (n=116)				Female (n=72)			Male (n=44)			
	HOCO	RCO	Control	*P*[2]	HOCO	RCO	Control	HOCO	RCO	Control	*P*[a]
Insulin (μIU/ml)	16.5 ± 2.4	16.4 ± 2.4	16.8 ± 2.4	0.89	16.1 ± 2.5	15.7 ± 2.5	16.2 ± 2.5	16.9 ± 2.6	17.2 ± 2.6	17.4 ± 2.6	0.28
Glucose (mg/dl)	93.9 ± 1.1	93.3 ± 1.1	94.5 ± 1.1	0.08	93.7 ± 1.3	92.9 ± 1.3	94.0 ± 1.3	94.1 ± 1.6	93.7 ± 1.5	5.3 ± 1.5	0.80
Fructosamine (μmol/l)	224.3 ± 2.3	223.8 ± 2.3	224.7 ± 2.3	0.63	220.9 ± 2.5	219.5 ± 2.5	220.6 ± 2.5	227.8 ± 2.9	228.2 ± 2.9	228.7 ± 2.9	0.63
HOMA-IR	3.89 ± 0.57	3.84 ± 0.57	4.00 ± 0.57	0.72	3.81 ± 0.59	3.70 ± 0.59	3.89 ± 0.59	3.96 ± 0.64	3.98 ± 0.64	4.10 ± 0.64	0.31
HOMA-β	203.7 ± 31.1	208.3 ±31.1	198.9 ± 31.1	0.45	196.4 ± 31.9	193.3 ±31.9	194.6 ± 31.9	211.1 ± 33.5	223.3 ±33.5	203.1 ±33.5	0.43

[1]All data represent the endpoint measures and are presented as least-squares means ± SEMs. HOCO: high-oleic acid canola oil; HOMA-β: homeostatic model assessment of β-cell function; HOMA-IR: homeostatic model assessment of insulin resistance; MUFA: monounsaturated fatty acids; RCO: regular canola oil.
[2]SAS PROC MIXED with repeated measure procedure was used to assess the effect of MUFA consumption on the reported glycemic control-related measures, using participants as a repeated factor. Treatment, sex, age, and genotype were used as fixed effects. Random effects were treatment sequence, clinical site, and participants. $P<0.05$ was considered significant. *P*: the difference between the treatments in the overall population. *P*[a]: *P* of gender-by-treatment interaction.

Table 3: Characteristics of Participants at the Baseline of Dietary Intervention[1]

Characteristic	Total (n=101)	Female (n=60)	Male (n=41)	*P*[2]
Age (years)	43.31 ± 1.29	45.65 ± 1.64	39.88 ± 1.98	0.0271
Ethnicity (n)				-
Caucasian	74	45	29	
African	4	3	1	
Asian	8	4	4	
Hispanic	3	1	2	
Others	12	7	5	
Waist circumference (cm)	103.8 ± 1.3	100.8 ± 1.6	108.3 ± 1.9	0.003
Body weight (kg)	89.76 ± 1.88	83.05 ± 2.21	99.59 ± 2.67	<.0001
BMI[3] (kg/m^2)	31.12 ± 0.53	30.99 ± 0.69	31.31 ± 0.84	0.7675
Systolic BP (mmHg)	118.6 ± 1.3	117.8 ± 1.7	119.7 ± 2.0	0.4647
Diastolic BP (mmHg)	78.10 ± 1.08	77.47 ± 1.41	79.00 ± 1.70	0.4915
Total cholesterol (mmol/L)	5.19 ± 0.09	5.21 ± 0.12	5.15 ± 0.14	0.7369
Triglycerides (mmol/L)	1.55 ± 0.07	1.46 ± 0.09	1.67 ± 0.11	0.1523
HDL-cholesterol (mmol/L)	1.35 ± 0.04	1.46 ± 0.04	1.20 ± 0.05	0.0003
LDL-cholesterol (mmol/L)	3.13 ± 0.08	3.09 ± 0.10	3.19 ± 0.12	0.5063
Glucose (mg/dl)	94.0 ± 0.7	93.8 ± 1.1	94.1 ± 1.3	0.8271
Insulin (μIU/ml)	14.21 ± 0.88	13.58 ± 1.14	15.12 ± 1.38	0.3908
HOMA IR	3.89 ± 0.27	3.71 ± 0.35	4.15 ± 0.43	0.4283
HOMA-β	196.5 ± 10.5	189.9 ± 13.6	206.1 ± 16.4	0.4508

[1]All values are means ± SEMS unless otherwise specified.

[2]SAS PROC MIXED procedure was used to assess the inter-sex differences, $P<0.05$ was considered significant. SAS PROC MEANS was used to determine the mean characteristics of the overall population.

[3]BP: blood pressure; BMI: body mass index; HDL: high-density lipoprotein; LDL: low-density lipoprotein; HOMA-β: homeostasis model assessment of beta-cell function; HOMA-IR: homeostasis model assessment of insulin resistance.

Table 4: Fasting Insulin, Glucose, and Homeostatic Model Assessment of Insulin Sensitivity and Β-Cell Function According to Dietary Intervention and Selected Polymorphisms[1]

Gene, SNP	Allele (n)	Insulin (μIU/ml)			Glucose (mg/dl)			HOMA-IR			HOMA-β		
		High MUFA	Low MUFA	*P*[2]	High MUFA	Low MUFA	*P*	High MUFA	Low MUFA	*P*	High MUFA	Low MUFA	*P*
ADIPOQ	CC (61)	16.5 ± 2.3	17.0 ± 2.3		93.4 ± 1.3	94.7 ± 1.3		3.8 ± 0.6	4.1 ± 0.6		208 ± 29	199 ± 29	
rs266729	CG (30)	15.9 ± 2.5	15.1 ± 2.6	0.42	91.7 ± 1.6	90.9 ± 1.7	0.08	3.6 ± 0.6	3.4 ± 0.6	0.29	208 ± 31	211 ± 32	0.74
	GG (10)	17.6 ± 3.2	17.8 ± 3.3		95.4 ± 2.6	97.3 ± 2.8		4.3 ± 0.8	4.4 ± 0.8		194 ± 39	184 ± 41	
ADRB2	CC (42)	16.0 ± 2.4	16.3 ± 2.4		94.7 ± 1.4	95.3 ± 1.5		3.8 ± 0.6	3.9 ± 0.6		184 ± 28	178 ± 29	
rs1042714	CG (41)	17.4 ± 2.4	17.3 ± 2.4	0.84	93.5 ± 1.4	94.0 ± 1.5	0.82	4.1 ± 0.6	4.1 ± 0.6	0.79	215 ± 28	209 ± 29	1.00
	GG (18)	15.2 ± 2.8	15.6 ± 2.8		88.6 ± 2.0	90.1 ± 2.1		3.4 ± 0.7	3.5 ± 0.7		235 ± 33	233 ± 34	
FABP1	CC (10)	17.9 ± 3.2	16.7 ± 3.3‡		97.6 ± 2.7	95.3 ± 2.8		4.5 ± 0.8	4.3 ± 0.9*		180 ± 39	171 ± 41	
rs2241883	TC (45)	15.5 ± 2.4	16.0 ± 2.4	0.14	92.2 ± 1.4	93.4 ± 1.4	0.06	3.6 ± 0.6	3.7 ± 0.6	0.08	203 ± 29	197 ± 30	0.95
	TT (46)	17.0 ± 2.4	17.2 ± 2.4		92.9 ± 1.4	93.9 ± 1.4		3.9 ± 0.6	4.0 ± 0.6		216 ± 29	212 ± 30	
FTO	AA (18)	18.6 ± 2.9	17.7 ± 2.9		91.2 ± 2.1	92.3 ± 2.2		4.2 ± 0.7	4.0 ± 0.7		268 ± 36	232 ± 37	
rs9939609	AT (39)	15.5 ± 2.5	15.3 ± 2.5	0.76	94.5 ± 1.5	95.3 ± 1.6	0.84	3.7 ± 0.6	3.7 ± 0.6	0.84	177 ± 32	170 ± 33	0.38
	TT (44)	16.5 ± 2.5	17.4 ± 2.5		92.7 ± 1.5	93.2 ± 1.5		3.8 ± 0.6	4.1 ± 0.6		210 ± 31	218 ± 32	
IRS1	CC (50)	16.2 ± 2.3	15.7 ± 2.4		92.5 ± 1.4	92.8 ± 1.4		3.8 ± 0.6	3.6 ± 0.6		204 ± 28	192 ± 29	
rs2943641	CT (42)	17.1 ± 2.4	18.3 ± 2.4	0.13	93.9 ± 1.4	95.2 ± 1.5	0.72	4.0 ± 0.6	4.4 ± 0.6	0.13	213 ± 29	218 ± 29	0.27
	TT (9)	13.8 ± 3.3	13.1 ± 3.4		92.7 ± 2.8	93.0 ± 3.0		3.1 ± 0.9	2.9 ± 0.9		173 ± 40	161 ± 43	
IRS1	AA (49)	15.4 ± 2.3	14.6 ± 2.3		92.8 ± 1.4	93.1 ± 1.4		3.6 ± 0.6	3.4 ± 0.6		194 ± 28	181 ± 29	
rs7578326	AG (45)	17.7 ± 2.3	19.2 ± 2.3	0.01	93.7 ± 1.4	94.8 ± 1.5	0.87	4.2 ± 0.6	4.6 ± 0.6	0.02	221 ± 28	228 ± 29	0.03
	GG (7)	13.6 ± 3.5	11.8 ± 3.6*		91.9 ± 3.2	92.5 ± 3.3		3.1 ± 0.9	2.6 ± 0.9‡		178 ± 43	151 ± 46*	

Table 4: Continued

Gene, SNP	Allele (n)	Insulin (μIU/ml)			Glucose (mg/dl)			HOMA-IR			HOMA-β		
		High MUFA	Low MUFA	*P*	High MUFA	Low MUFA	*P*	High MUFA	Low MUFA	*P*	High MUFA	Low MUFA	*P*
PLIN rs894160	CC (47)	16.4 ± 2.4	16.5 ± 2.4		94.2 ± 1.4	94.8 ± 1.5		3.8 ± 0.6	3.9 ± 0.6		196 ± 28	191 ± 29	
	CT (43)	16.9 ± 2.4	16.8 ± 2.4	0.57	92.0 ± 1.4	92.7 ± 1.5	0.78	3.9 ± 0.6	4.0 ± 0.6	0.55	222 ± 28	214 ± 29	0.88
	TT (11)	14.9 ± 3.1	16.1 ± 3.2		93.5 ± 2.5	95.0 ± 2.7		3.4 ± 0.8	3.8 ± 0.8		183 ± 37	185 ± 39	
PPARγ rs1801282	CC (78)	16.0 ± 2.3	16.3 ± 2.3		92.2 ± 1.0	92.7 ± 1.1		3.7 ± 0.6	3.8 ± 0.6		208 ± 28	205 ± 28	0.36
	CG (21)	17.4 ± 2.7	17.1 ± 2.8	0.54	95.5 ± 1.8	96.7 ± 1.9	0.72	4.1 ± 0.7	4.1 ± 0.7	0.66	201 ± 33	187 ± 34	
	GG (2)	21.3 ± 5.9	21.1 ± 6.1		105.0 ± 5.6	107.7 ± 5.9		5.6 ± 1.6	5.6 ± 1.6		193 ± 71	176 ± 77	
TCF7L2 rs7903146	CC (48)	15.8 ± 2.4	15.7 ± 2.4		92.1 ± 1.3	92.2 ± 1.3		3.6 ± 0.6	3.6 ± 0.6		198 ± 29	200 ± 30	
	CT (46)	17.0 ± 2.4	17.7 ± 2.4	0.95	94.2 ± 1.3	95.5 ± 1.4	0.31	4.0 ± 0.6	4.3 ± 0.6	0.97	215 ± 30	204 ± 30	0.38
	TT (7)	17.8 ± 3.6	16.0 ± 3.7		93.9 ± 3.1	95.5 ± 3.2		4.2 ± 0.9	3.7 ± 1.0		209 ± 44	189 ± 47	

[1]All data represent the endpoint measures and are presented as least-squares means ± SEMs. n=101 participants. *ADIPOQ*: adiponectin; *ADRB2*: adrenoceptor beta-2; *FABP1*: liver fatty acid binding protein; *FTO*: fat mass and obesity-associated gene; HOMA-β: homeostatic model assessment of b-cell function; HOMA-IR: homeostatic model assessment of insulin resistance; *IRS1*: insulin receptor substrate-1; MUFA: monounsaturated fatty acids; *PLIN*: perilipin-1; *PPARγ*: peroxisome proliferator-activated receptor gamma; SNP: single nucleotide polymorphism; *TCF7L2*: transcription factor 7-like 2.

[2]SAS PROC MIXED with repeated measure procedure was used to assess the effect of gene-MUFA interactions on glycemic homeostasis and HOMA indices, using participants as a repeated factor. Treatment, sex, age, and genotype were used as fixed effects. Random effects were treatment sequence, clinical site, and participants. *P* <0.05 was considered significant. *P*-values presented in the table refer to overall gene-diet interaction. * Indicates significant difference between the consumption of high-SFA versus high-MUFA within the same genotype. ‡Indicates trend toward significance (p<0.07) between the consumption of high-SFA versus high-MUFA within the same genotype.

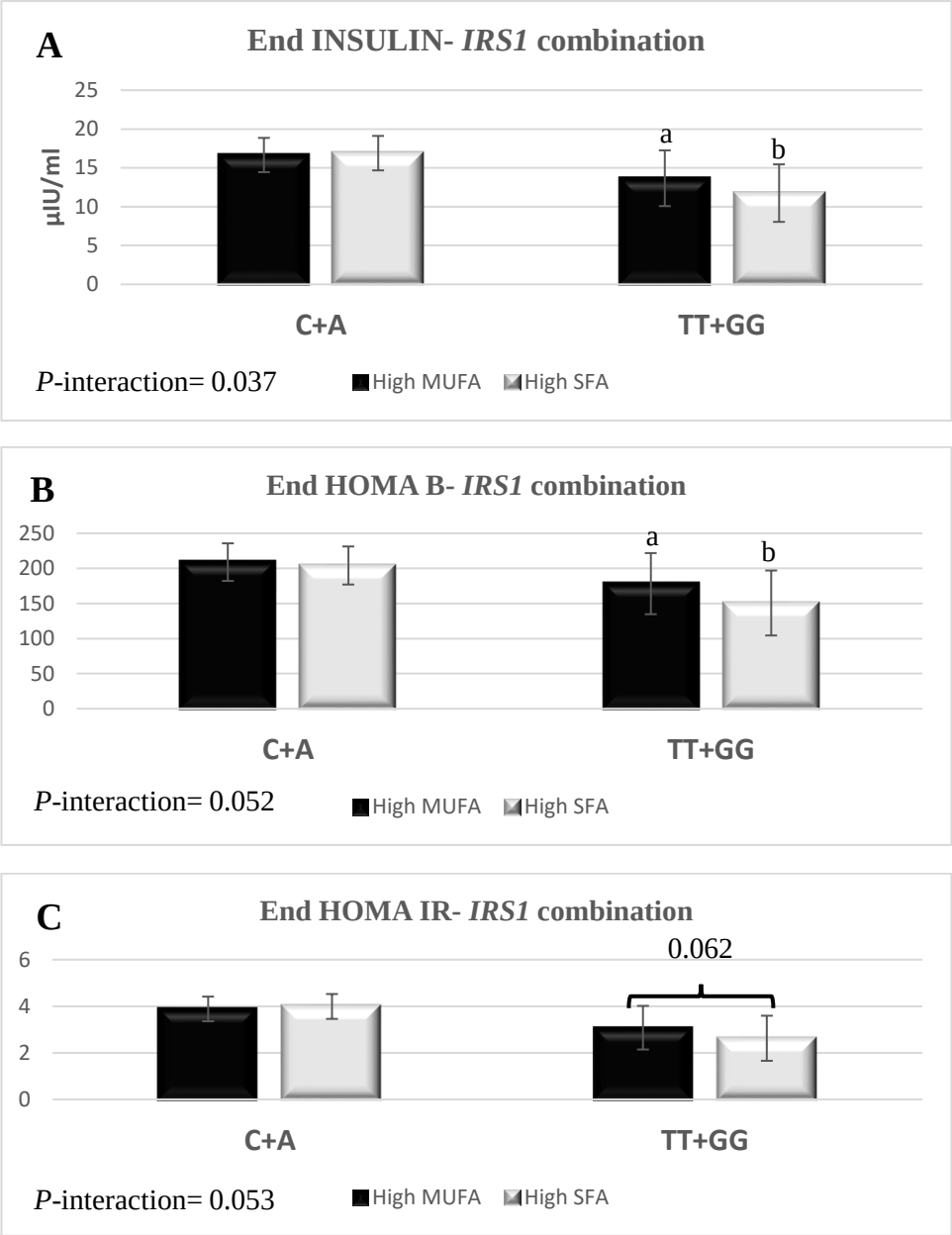

Figure 1: End-point responses of insulin (A), HOMA-β (B), and HOMA-IR (C) to dietary MUFA by *IRS1* rs2943641 + rs7578326 combination. SAS PROC MIXED with repeated measure procedure used to assess the effect of gene-MUFA interactions on IR and glycemic response, using participants as a repeated factor. Treatment, sex, age, and genotype were used as fixed effects. Random effects were treatment sequence, clinical site, and participants. P <0.05 was considered significant. Values are least-squares means ± SEMs. Labeled bars with different lowercase letters significantly differ, P <0.05. HOMA: homeostasis model assessment of insulin resistance (IR) and cell function (β); *IRS1*: insulin receptor substrate-1 gene; *IRS1* combination: rs2943641-C/T and rs7578326-A/G; MUFA: monounsaturated fatty acid; SFA: saturated fatty acid. Frequency of SNP combination (n=99): C+A=93% and TT+GG=7%.