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Nanocellulose as a Functional Ingredient in the Management of Metabolic Syndrome: A Review

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ABSTRACT

Alternative treatments in the management of metabolic syndrome are required because multiple drugs for individual components was found to have negative side effects on other components. Functional ingredients, particularly fiber, has shown great benefits in improving metabolic syndrome. Nanocellulose is a novel type of fiber, derived from cellulose through various processes that result in a nanoscale fiber with the dimension below 100 nm. Its smaller size brought improvements to the physicochemical properties of cellulose and consequently its biological activities. Nanocellulose appear to exhibit distinct functional activities that affect various processes in the gastrointestinal tract, including interference in lipid and carbohydrate digestion and reinforcement of gut microflora. These properties may ameliorate abdominal obesity, dyslipidemia, hyperglycemia, and high blood pressure through similar mechanisms of both soluble and insoluble fibers. In this review, we first introduce nanocellulose and its particular characteristics that makes it separate from cellulose. With the limited studies available, we try to go in depth into its activity in the gastrointestinal tract followed by the possible implications of those functional properties on health, especially on the components of metabolic syndrome. Lastly, we discuss the potential applications and advantages of incorporating nanocellulose in functional food for the management of metabolic syndrome.

Keywords : nanocellulose; fiber; metabolic syndrome; functional food

BACKGROUND

As the numbers of noncommunicable disease cases such as cardiovascular disease and diabetes continue to increase, it brings about the rise of a discernible condition now commonly known as metabolic syndrome. Metabolic syndrome is generally characterized by at least three of the following signs, i.e. high blood glucose or insulin levels, high blood pressure, abdominal obesity, and abnormal lipid profile, specifically elevated triglycerides and low high-density lipoprotein (HDL) concentration.[1] The prevalence of metabolic syndrome is estimated to be about a quarter of the world's population, although the reported data might vary based on the criteria used and the characteristics of the studied population.[2] Approximately 12-49% of the Asian population were estimated to have metabolic syndrome, with women and urban residents having the higher proportion of cases.[3] In Indonesia, it was reported that about 28% of men and 46% of women have metabolic syndrome.[4] These numbers may continue to rise in the upcoming years especially with the growingly common dietary pattern of consuming high calorie food with low fiber intake.[5,6]

The general guidelines in the management of metabolic syndrome consist of medication and lifestyle changes, specifically regular physical activity and diet interventions. Medications given typically consist of multiple drugs that treats each component separately with common side effects that may adversely impact the other components.[7–10] The use of statin to reduce high cholesterol levels, for example, increased the risk for diabetes by 46% due to impaired insulin sensitivity and secretion.[9] Therefore, there is a need for alternative treatments that can improve various attributes of metabolic syndrome without causing much side effects. Functional food and nutraceuticals are viewed as promising options as they possess favorable effects on metabolic homeostasis.[11,12] Fiber, in particular, is a great functional ingredient as it has been proven to significantly decrease the risks for metabolic syndrome[13] through improving glucose homeostasis[14,15], reducing the absorption of triglycerides[16,17], and lowering body fat[18,19]. Traditionally, fiber can be obtained by eating vegetables and fruits, but the development of food technology has broadened our options and even enabled us to modify the content of functional ingredient in food to produce food with more health-

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promoting benefits.[20–22] The emergence of nanotechnology further aided the growth of functional food by introducing various nanomaterials including nanoscale fibers such as nanocellulose.

Nanocellulose is a nanomaterial derived from cellulosic materials with at least one dimension less than 100 nm. There is a growing interest for the application of nanocellulose as it has exhibited many attractive qualities, such as minimal toxicity, low density, biodegradability, and compatibility with biological tissue.[23–26] Aside from the general characteristics of cellulose, nanocellulose possesses larger surface area, higher viscosity, better dispersion, and most interestingly, gelling properties that resemble soluble fiber.[26,27] These characteristics may affect its behavior in the gastrointestinal tract and how it interacts with other nutrients and intestinal bacteria. Simulated digestion of starch along with nanocellulose revealed that 1% of nano-fibrillated cellulose impaired 26.6% of glucose release.[28] Other studies reported that nanocellulose significantly reduced the absorption of triglycerides, free fatty acids, and saturated fat.[29,30] Furthermore, nanocellulose significantly improved the production of short chain fatty acids during intestinal fermentation, increasing it by over 200%.[31,32] These findings imply hypoglycemic and hypolipidemic effects as well as other potential health benefits of nanocellulose that may become beneficial in the management of diseases including metabolic syndrome.

So far, only a few studies have investigated the application of nanocellulose in terms of its functional activities for health. DeLoid et al reported lipid-lowering benefits with reduced serum triglycerides up to 36% in rats given nanofibrillated cellulose, whereas Lu et al discovered significant decrease in plasma cholesterol from nanocrystal cellulose gavage in mice.[29,33] Previous reviews have detailed the use of nanocellulose in cosmetics, paper, textile, biomedicine, and food industry[25,27,34,35], but it appears that not many have discussed its potential as a functional substance for health and disease management. Therefore, this review article will discuss the potential of nanocellulose as a functional ingredient for metabolic syndrome based on its physicochemical properties and its effects on processes that may affect metabolic biomarkers, the health implications it may entail, and the potential application for nanocellulose as functional food.

CELLULOSE AND NANOCELLULOSE

Cellulose is the most widely available and abundant biopolymer on Earth, most well-known as the structural and reinforcement component in cell wall of plant.[24] Approximately 7.5 x 10^{10} tons of cellulose are produced every year[36] by not only plants but also bacteria, fungus, tunicates, and some species of invertebrates and amoeba.[26] Structurally, cellulose is a linear polysaccharide consisting of glucose monomer units that carry three hydroxyl groups each.[24] Each cellulose fiber is formed by a bundle of cellulose crystals linked along microfibrils that are tightly connected by hydrogen bonds and enclosed with matrix components namely hemicellulose, pectin, and lignin. About a quarter of dietary fiber in grains and fruits and one third in vegetables are made up of cellulose.[37] As an insoluble fiber, cellulose increases fecal volume and undergoes fermentation by intestinal microflora which produces beneficial byproducts like short-chain fatty acids.[38]

Using certain methods, cellulose can be broken down into its nanoscale components or commonly known as ‘nanocellulose’. Nanocellulose generally describes various nanomaterials derived from cellulose with at least one dimension in the nanometer range.[27] There are two major methods to obtain nanocellulose: top-down and bottom-up.[27] Top-down methods involve enzymatic or chemical or mechanical treatments to isolate nanocellulose from lignocellulosic residues. The bottom-up method involve production of nanocellulose from glucose by bacteria. These methods produce the three main types of nanocellulose, namely nanocrystals cellulose (NCC), nanofibers cellulose (NFC), and bacterial nanocellulose (BNC).

Both NCC and NFC are produced through top-down methods from materials like wood, potato tuber, sugar beet, seed fiber, grasses, wheat straw, bark, marine animals (tunicate), and algae.[24,25] The extraction of NCC begins with acid hydrolysis to remove amorphous regions of the material. Afterwards, free acidic molecules and impurities are removed using dilution and washing along with centrifugation and extensive dialysis. Lastly, the NCC particles are stabilized as uniform suspension by sonication. The diameter of NCC ranges from 5-70 nm with various lengths depending on its source material, starting from 100-500 nm for plant cellulose and 100 nm to several µm for algae and tunicate cellulose.[24] NCC appears as rigid, long needle-like nanoparticles under electron microscope observations.[39] NCC carries a negative surface charge.

On the other hand, NFC is mostly isolated through mechanical treatments such as high-pressure homogenization, grinding, waring blender, or microfluidizer.[26] These treatments are meant to break the side aggregations and delaminate the individual microfibrils. Pretreatments like 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) oxidation are often applied beforehand to weaken interfibrillar hydrogen bonds, to reduce the energy consumption needed in NFC extraction, and to convert primary hydroxyl groups of cellulose to carboxyl groups.[26,40] NFC has a diameter of 5-60 nm and length of several µm. NFC has a more flexible structure than NCC due to its alternating crystalline and amorphous regions.[24]

Unlike NCC and NFC, BNC is extracted using the bottom-up method where bacteria synthesize cellulose in a pure form, requiring no treatments to remove impurities or contaminants. Glucose chains and cellulose molecules are generated inside the bacteria and then extrude out through pores on the cell surface, before assembling and further aggregating into nanofibrils.[26,41] Bacteria that can be used to produce BNC are *Aerobacter, Achromobacter, Agrobacteriuj, Pseudomonas, Rhizobium, Salmonella, Zooglea,* and *Escherichia*, but so far only *Gluconacetobacter* can produce BNC for commercial use.[42] The diameter of BNC is around 20-100 nm.[24]

General physicochemical characteristics of cellulose was found to be improved in nanocellulose. Due to its smaller size, nanocellulose has larger specific surface area which indicated more exposure of free hydroxyl groups on the surface. These hydroxyl groups develop hydrogen bonds that give nanocellulose a reactive surface for associations with substances.[43] This was supported by the findings of an inverse relationship between cellulose particle size with water holding capacity, swelling capacity, and oil-holding capacity.[31,44] Similarly, Dubey at al reported that water holding-capacity and swelling capacity of nanocellulose were, respectively, 7.3 times and 9 times higher than cellulose.[32] Swelling capacity can affect the fermentation of fiber, further proven by another study where nanocellulose has higher fermentability than cellulose.[31] Apart from that, nanocellulose has low toxicity as well as good biocompatibility and biodegradability.[45,46] Cellulose is generally found to be biocompatible with minimum to no adverse effects, but it is not highly biodegradable for the human body due to the lack of cellulolytic enzymes. Although still not readily degradable, nanocellulose were biodegraded at a relatively faster pace than cellulose because of its higher surface area.[47] Nanocellulose also has shear-thinning properties where they cause decreased viscosity in fluid under shear force, making it beneficial for the processing and storage of fluids.[48] Another interesting property is the capability of nanocellulose to accumulate at oil-water interface in emulsions to form a physical barrier that prevents the emulsion droplets from coalescing.[49] Other properties of nanocellulose include high aspect ratio, high stiffness and tensile strength, low density, rheological properties, barrier properties, and mechanical reinforcement.[25] The stark differences in properties between cellulose and nanocellulose clearly imply distinct biological activities.

FUNCTIONAL ACTIVITES OF NANOCELLULOSE

A food or substance is considered 'functional' when it evokes positive biological effects that provides protection from diseases through mechanisms other than fulfilling nutritional needs.[12] The functional activities of fiber are generally determined by the type of fiber, either soluble or insoluble fiber.[38] Soluble fiber forms viscous gels in the stomach, resulting in delayed gastric emptying and consequently reduced glycemic response[50]. Insoluble fiber inflates the volume of food bolus, increases gastrointestinal transit time, and improves intestinal function, causing reduced absorption of triglyceride and cholesterol.[38,51] All types of dietary fiber undergo fermentation in the large intestine, directly influencing the balance of gut microbiome and determining the generated fermentation products.[51] Since nanocellulose has its own distinguished properties, it is possible that it might not entirely have the exact same effects as cellulose on the processes in the gastrointestinal tract and its metabolic implications. It is important to identify the types of functional activities nanocellulose possess to determine the exact health benefits it may provide as well as the proper application methods to sustain its functional activities. The following is an overview of studies that examined the potential functional activities of nanocellulose, especially in the gut.

Effects on Lipid Digestion and Absorption

As with other dietary fibers, nanocellulose has shown potential hypolipidemic activities in simulated gastrointestinal digestion models. The addition of nanocellulose resulted in slower lipid digestion and lower lipolysis rates.[29,52–56] DeLoid et al reported that NFC significantly reduced fatty acid hydrolysis up to 48.4%.[29] Similarly, another study used NCC to stabilize emulsions and it generated less free fatty acids compared to gum Arabic-coated emulsions during simulated digestion.[52] Sarkar et al also found that the use of 3% NCC reduced the rate and degree of lipid digestion, going as far as to 8 times and 3 times lower, respectively.[56] Increasing the concentration of nanocellulose appeared to enhance the inhibition effect as the lowest amount of hydrolysis products was mostly found at the highest concentration studied, around 1-3% dry weight.[52,53,56] In addition, nanocellulose exhibited excellent cholesterol adsorption capacity, reportedly adsorbing 99.99% (8.5 mg/dry weight fiber) of cholesterol, which was significantly higher than cellulose (84%, 7.1 mg/dry weight fiber).[53] Furthermore, a study using an *in vitro* triculture small intestinal epithelial model revealed that NFC reduced the absorption of triglyceride by 35% and free fatty acids by 54%.[29] Mackie et al also found that NCC caused significantly less absorption of saturated fat.[30]

There are several mechanisms underlying the effects of nanocellulose on lipid digestion. Considering its larger surface area and high water-holding capacity, nanocellulose can reduce free water content in digesta, thereby increasing the viscosity.[54,55] The viscosity was particularly increased after gastric digestion, most likely due to high ionic strength that compressed the electric double layer and allowed NCC to interact through van der Waals forces and hydrogen bonds.[54,57] Highly viscous digesta resulted in slower digestion and lower absorption of lipid as it prevented the bulk diffusion across intestinal lumen.[33]

Examinations under various microscopy systems demonstrated that nanocellulose formed a surrounding structure that encapsulates and attaches to fat droplets, thus limiting the adsorption of lipase and preventing the hydrolysis of fat droplets.[29,52,56] NCC formed a strong, rigid, densely packed coating on the surface of fat droplets.[52] Closer examination revealed that nanocellulose bridged together small emulsion droplets in a raspberry-like floc that was similar with emulsion microgel particles.[56] Similarly, NFC were found to form a honeycomb-like lattice with fat droplets attached to its structure.[29] This was also confirmed by Winuprasith et al who reported that NFC may have adsorbed to the surfaces of several fat droplets at once, creating a tight cluster bond.[55] Although it has not been proven yet in the current studies, high nanocellulose concentrations were assumed to ascertain lipid droplets becoming wholly surrounded by cellulose nanocrystals, leaving no uncovered gaps that are accessible for lipase or bile salts.[52,56]

Another mechanism involved the sequestration of bile salts by nanocellulose through hydrogen binding with hydrophilic groups.[52,56] Liu et al found that NFC at 2% (w/w) caused significantly lower bile acid diffusion rate than control (5.48% vs 8.0%) with a bile acid retardation index of 27, owing to higher viscosity from the binding of cellulose nanofibrils to bile acid.[53] Fiber-bound bile acid is excreted through the feces, as confirmed by Lu et al that observed higher total bile acid levels in fecal matter of rats fed with NCC.[33] The decrease of available bile acid hindered the displacement of interfacial materials at the surface of fat droplets, further preventing the adsorption of lipase-colipase complexes on fat droplets.[29] Insufficient bile acid would also stimulate the utilization of cholesterol for bile acid production, consequently reducing lipid and cholesterol absorption.[53] Furthermore, bile acid is necessary for the solubilization and removal of lipolytic products from the surface of fat droplets. Accumulation of such products like free fatty acids and monoacyglycerols would hamper further digestion processes such as hydrolysis by pancreatin.[29,55]

Effects on Carbohydrate Digestion and Absorption

Carbohydrate digestion occurs starting from the mouth and all the way into the intestines, meaning that there are many possible sites where nanocellulose can affect the process. A linear relationship was found between the addition of NFC and the decrease of *in vitro* glucose diffusion, with significant differences observed starting from 0.5% of NFC.[28,58] As the concentration rose from 0 to 2%, NFC steadily reduced glucose diffusion rates from 22.4 to 9.5 µmol/minute.[28] Retardation effect of fiber on glucose absorption in jejunum was quantified using glucose dialysis retardation index (GDRI) and 2% nanocellulose recorded a maximum value of 50%, much higher than fiber fractions from wheat bran (34.8%) and oats (29.7%).[59] Moreover, the digestion of starch with 1.1% NFC revealed significantly reduced glucose release.[28]

Nanocellulose also exhibited inhibitory effects on enzymes linked with carbohydrate digestion, particularly amylase. Amylase is an enzyme produced by saliva and pancreatic juice and functions to hydrolyze starch into maltose and glucose. The rate of hydrolysis by amylase declined with 1-2% of NFC, resulting in only approximately 70% of glucose was released after 6 hours of digestion.[28] Likewise, Ji et al reported that an inverse relationship between the activity of α-amylase and NCC concentration. Results from infrared spectroscopy and circular dichroism analysis suggested changes in the structure of α-amylase, confirming that NCC affected its activity by associating with α-amylase, possibly through weak non-covalent interactions and hydrogen bonds.[60]. Similar effects were found with glucoamylase. It was supposed that the hydroxyl groups in NCC migrated into protein's interior and form hydrogen bonds with atoms in the backbone.[60]

Glucose diffusion rates are determined by solution viscosity and the amount of glucose adsorbed in the fiber. Higher nanocellulose concentrations result in higher viscosity, causing an increase in the thickness of unstirred water layer next to the intestinal membrane and eventually making it harder for nutrients to be absorbed.[28] Furthermore, nanocellulose could bind three times more glucose than cellulose, reportedly 35.6% compared to only 12.8% of 200 mM glucose.[28] This effect was still prominent even at low glucose concentrations.[28] Nsor-Atindana et al also reported suppressed glucose release and diffusion in starch digested with NCC suspension, attributing it to the increased viscosity since the inhibition effect was higher in more viscous samples containing smaller-sized NCC particles.[61]

Effects on Gut Microbiome

For the last decade, gut microbiome has been a great interest of study for its vital role in maintaining various bodily functions. Numerous studies showed that compromised gut bacteria contribute to the development of various diseases, including metabolic syndrome.[62] The consumption of prebiotics such as dietary fiber was reported to help improve gut barrier integrity and microbiota balance, thus it was reasonable to assume that nanocellulose could do the same.[62]

Several studies have described the beneficial effects of nanocellulose on gut microbiome and its metabolic products.[31,32] Using pH value as the index of fiber fermentation, studies have found that the addition of nanocellulose reduced pH during fermentation, suggesting that short chain fatty acids were abundantly produced. The decline of pH was greater in nanocellulose with the least particle size.[31,32] Lower intestinal pH provides protection against pathogenic bacteria and stimulates the growth of beneficial gut microbiome such as *Lactobacillus* and *Bifidobacterium*. This result was supported by Lopes et al who found that NFC may possess antibacterial activity against Gram-positive bacteria such as *Escherichia coli* but had no effect on *Lactobacillus reuteri.*[46]

Results from *in vitro* fermentation using human feces revealed that the number of *Bifidobacterium* bacteria significantly increased with 1% nanocellulose after 4-hour fermentation.[31] Interestingly, the bacteria count rose as the particle size decreased, with the smallest sized nanocellulose constantly having the highest number of bacteria throughout 24-hour fermentation. Accordingly, the amount of generated short-chain fatty acids was also size-dependent. A direct correlation was found between specific surface area of cellulose with the concentration of total short-chain fatty acids with R values of 0.837-0.988.[31] The same results were observed during *in vivo* experiment where faecal matter of rats orally fed with nanocellulose had significantly higher *Bifidobacterium* count and short-chain fatty acid concentrations in comparison to non-cellulose and microcellulose fed groups.[31]

Similarly, Dubey et al detected higher levels of acetate, propionate, and butyrate during simulated fermentation of reduced size cellulose in contrast to the original-sized one.[32] Acetate had the highest concentration, followed by propionate and lastly butyrate. In contrast, Nsor-Atindana et al reported butyrate as the highest, and then propionate and acetate.[31] However, their *in vivo* experiment revealed similar results as Dubey et al. The differences in short-chain fatty acid production may be attributed to the variation of intestinal microbiota profile among individuals.[32] Regardless, increased short-chain fatty acids appears to be the main mechanism for various health benefits provided by gut microbiome.

On the contrary, Khare et al reported possible adverse reactions on gut microbiome due to ingested nanocellulose. The gavage feeding of rats with 1% NFC resulted in decreased intestinal bacteria species, including *Caprococcus catus* that contributes to the production of short-chain fatty acids and *Bacteroides acififaciens* that is linked with increased insulin levels.[63] However, notable reduction of potential pathogenic and disadvantageous bacteria was also observed, such as *Tannerella* spp. that is known to stimulate foam cell formation.[63] More studies are required to be able to gain a complete picture of the effects of nanocellulose on gut microflora.

Health Implications of the Functional Activities of Nanocellulose on the Components of Metabolic Syndrome

The previous overview shows that nanocellulose clearly has functional impacts on various processes in the gastrointestinal tract. These impacts may amount to beneficial health effects in the body, including improvements of the components of metabolic syndrome.

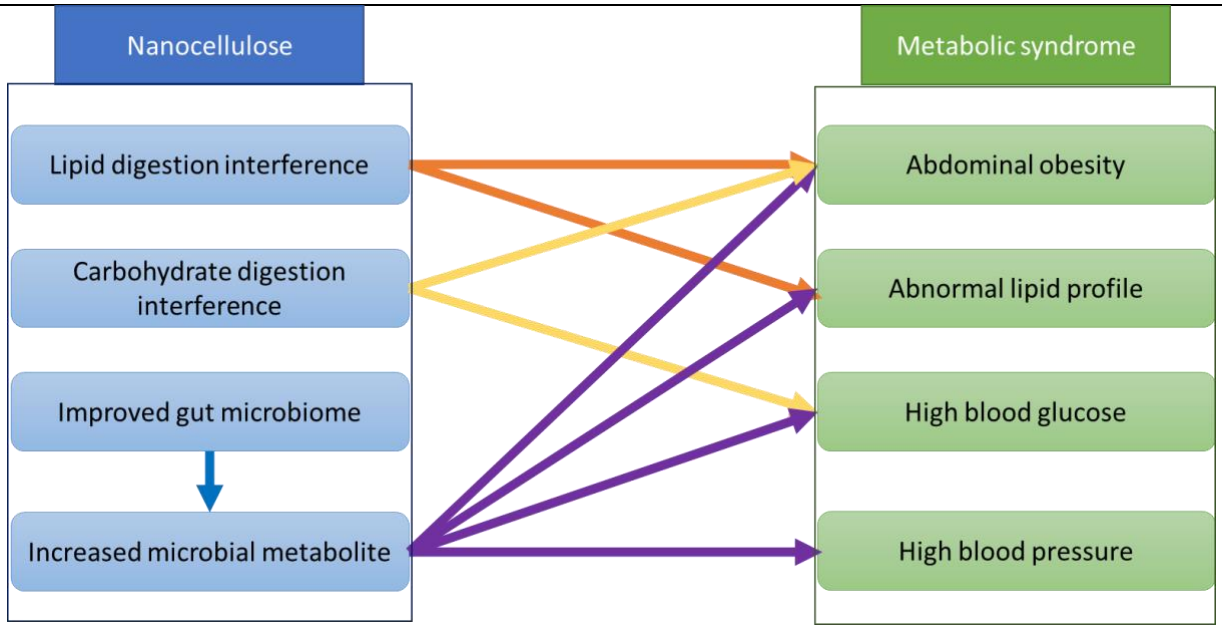
Abdominal obesity, commonly measured by waist circumference, serves as a practical screening tool for metabolic syndrome. It demonstrates the accumulation of fat in visceral tissues which eventually triggers the release of various peptides that contribute to the development of metabolic syndrome. The overload of fat storage arises from energy imbalance, mainly due to excessive energy consumption with fat being the highest contributor. Fiber is well-known to reduce energy intake and obesity by affecting digestion rate through increasing viscosity of digesta in the gut, lowering gastric-emptying rate and limiting digestive enzyme activities on nutrients.[50] As a result, the presence of nutrients in the intestines is prolonged and the absorption is delayed, leading to altered secretion of appetite control peptides namely glucagon-like peptide 1 (GLP-1) and cholecystokinin (CCK).[64] Reduced appetite and greater satiety result in lowered energy intake and eventually decreased waist circumference and body fat in the long term, as shown in previous studies.[18,65,66] Lambert et al provided pea fiber for 12 weeks and reported significant depletion in energy intake and body fat.[18] Similar digesta viscosity-enhancing effects were observed with nanocellulose during artificial digestion, suggesting that it would also have the same weight loss promoting effects as other fibers. Moreover, nanocellulose exhibits fat-entrapping capacity that is comparable to chitosan, which has been proven to achieve weight loss and diminished visceral fat even though subjects were not given specific diet restrictions.[67]

Another component of metabolic syndrome that may be ameliorated by nanocellulose is dyslipidemia. Dyslipidemia mostly occurs from a combination of high-fat diets with sedentary lifestyle, so a switch to low-fat with high fiber diets is commonly recommended to reduce elevated cholesterol and triglyceride levels.[68] Fiber demonstrates lipid lowering effects through several mechanisms.[69] First, it lowers overall energy intake by increasing satiety as previously explained. Secondly, it entraps bile acids and increases fecal excretion of bile acid and cholesterol, consequently decreasing bile acid re-absorption. Because of that, hepatic uptake of cholesterol is amplified to produce bile acids, thus reducing total cholesterol and low-density lipoprotein (LDL) cholesterol levels.[70] As mentioned, a similar fiber-bile acid relationship was observed with nanocellulose and further supported by animal studies that reported hypocholesterolemic effects. In a 28-day long study on ovariectomized rats, Lu et al found that NCC significantly decreased plasma total cholesterol and low-density lipoprotein cholesterol (LDL-C).[33] Several other studies affirmed that particle size reduction of cellulose improved its functional effects in lowering serum cholesterol in rats.[71,72] Further examination in animal studies revealed that NCC affected the expression of hepatic and ileac genes that are linked with cholesterol homeostasis. Further examination revealed that NCC caused the down-regulation of HMG-CoA reductase which is the rate-controlling enzyme for cholesterol biosynthesis, whereas mRNA levels of ASBT and IBABP that modulate intestinal absorption of bile acids were up-regulated.[33] This effect is most likely attributable to enhanced short-chain fatty acid production, especially butyrate.[73,74] This lipid-lowering capacity of nanocellulose would surely be beneficial in both the prevention and therapy of metabolic syndrome and other related illnesses like cardiovascular disease.

Furthermore, nanocellulose may have positive effects for glucose response by attenuating blood glucose levels using combined properties from both insoluble and soluble fibers. Insoluble fibers increase bulking of food matrix with its high water-binding capacity which leads to reduced glucose diffusion and suppressed increment of blood glucose.[75,76] Administration of insoluble barley fiber has been found to cause significantly ($p<0.01$) lower blood glucose levels in subjects with hyperglycemia when compared to placebo.[75] Soluble fibers delay gastric emptying and affects carbohydrate-related enzymes in the small intestine, resulting in lessened glucose and insulin response.[15,50] Both fibers also play a role in microbial metabolite production which includes various short-chain fatty acids that evoke the release of intestinal hormones linked with insulin and glycemic regulation.[50] All these mechanisms underlying hypoglycemic effects in the body were also observed with nanocellulose, thus indicating its possible efficacy in improving glycemic control. In addition, results from animal studies confirm the hypoglycemic capacity of nanocellulose. The administration of TEMPO-oxidized NFC with glucose and glycerol trioleate to mice resulted in decreased postprandial blood glucose, plasma insulin, and triglycerides.[77] Glucose-dependent insulinotropic polypeptide (GIP), which is involved in insulin secretion and fat metabolism, was also reduced.[78]

As for other parameters of metabolic syndrome, nanocellulose may cause betterment through stimulation of gut microflora and the production of short-chain fatty acids (SCFAs). SCFAs has been recognized as an important key for metabolic health as they are involved in numerous pathways that are linked with metabolic and inflammatory responses.[79,80] SCFAs, mostly acetate and propionate, modulate the expression of genes involved in blood pressure regulation. Propionate mediates GPR41 receptor which induces reduction of blood pressure.[81] Furthermore, SCFAs activate free fatty acid receptors (FFAR), specifically FFAR2, which is mainly activated by acetate and propionate, and FFAR3, which is activated by propionate and butyrate.[62] Both FFAR2 and FFAR3 trigger the secretion of intestinal hormones, such as GLP-1 that stimulates glucose-dependent insulin secretion and inhibits glucagon release, thus improving insulin resistance and overall glycemic control.[82] SCFAs were also found to reduce the expression of FXR which regulates triglyceride homeostasis.[17] That might be the plausible mechanism for lowered serum triglycerides when rats were provided heavy cream with 1.0% NFC. Serum triglycerides were curtailed by 36% at 1-hour post-gavage and the effect remained consistent after 2 hours.[29]

Altogether, the functional activities of nanocellulose appear to be beneficial for the treatment of metabolic syndrome. However, more research is needed to further verify this as some studies reported adverse effects instead. Andrade et al revealed no significant changes in the lipid profile nor blood glucose levels of rats fed with NFC for thirty days.[35] Another study examined the effect of oral NFC treatment in rats for 4-6 weeks, discovering that it caused negative effects instead, specifically dysregulation of glucose homeostasis, decreased lean body mass, and elevated body fat.[83] Generally, studies about the *in vivo* functional properties of nanocellulose are still limited and most are animal experiments while human studies are scarce.



Graphic 1. The link between the functional activities of nanocellulose with the components of metabolic syndrome that may be affected.

Potential Application of Nanocellulose in the Management of Metabolic Syndrome

Dietary changes are a major part of the lifestyle modification required for the prevention and treatment of metabolic syndrome. Increasing fiber is one of the most prescribed modifications as it has repeatedly shown beneficial impacts on individual components of metabolic syndrome. Besides the wholefood sources of fiber, various innovations through chemical, thermal, and enzymatic processing have been made to expand the utility and application of fiber.[84] Nanocellulose is a result of those innovations and with its small size and improved functionality, it has great versatility in its application to support the fulfilment of dietary recommendations to ameliorate metabolic syndrome. There are two plausible ways to use nanocellulose as a therapeutic ingredient: as a dietary supplement or as a food additive to make functional food. Dietary fiber supplements are commonly found nowadays and are mostly presumed to be able to give the same health benefits as whole fibers but has a high risk of causing gastrointestinal distress due to significant differences in stool viscosity.[85,86] However, further studies are still required to verify if the same impact would be found with nanocellulose supplements.

The use of nanocellulose as an additive in food would most likely reduce the potential gastrointestinal disturbances and have other benefits both on bodily health and the quality of food. With its high water-holding capacity, nanocellulose can be used as an additive to produce low-calorie food. Most processed food products have high energy density because of their low water content, thus increasing the water content would lead to reduced energy density.[87] Incorporating nanocellulose may be used to lower energy density of processed foods up to < 1.6 kcal/g.[88] Furthermore, nanocellulose has shown great potential as an emulsion stabilizer, making it suitable for application in commonly high-calorie emulsion food products such as dressings, toppings, sauces, puddings, and others.[27,49] Several patents have been established for the use of nanocellulose to replace fats completely or partially in toppings, fillings, gravies, etc.[27,89] Aside from reducing initial energy content, the addition of NFC to high-fat food models has been proven to exhibit strong interference effect on fat digestion.[29] The effect was observed in food models with various fat types, containing varied amounts of saturated and unsaturated fat as well as fatty acid profiles. The highest inhibition effect (48.4% reduction) was found in heavy cream and it was postulated that fatty acid chain length played a big role in determining the extent of interference by nanocellulose.[29] This finding suggested that nanocellulose can be used as an additive in high-fat food to counter the negative impacts of excessive fat intake.

On top of that, nanocellulose may improve the quality of specific food contents and enhance its functional properties; in this case, it is starch. Starchy food is prone to retrogradation which causes detrimental effects on its quality, but the addition of NCC has been shown to inhibit both long-term and short-term retrogradation.[90] Ji et al mixed NCC with various types of gelatinized starch and discovered that it led to significantly higher resistant starch content and lower digestible starch content. The resistant starch content rose ~30% for corn, ~23% for pea, and ~20% for potato starch.[60] Resistant starch is the part of starch that cannot be digested by enzymes in the small intestine, but is fermented by gut microbiota in the large intestine. Various health benefits can be obtained from consuming resistant starch, including improved glycemic and

insulinemic response, improved blood lipid profile, reduced fat accumulation, increased satiety, and prebiotic effects.[91] These physiological effects are certainly favorable in the treatment of metabolic syndrome.

As a source of fiber, nanocellulose can be incorporated into food to provide the benefits of increased fiber consumption. To date, the main challenge in supplementing fiber in food is the noticeable effect on the appearance, taste, texture, and flavor of food.[92] These concerns are no longer found in the application of nanocellulose, particularly BNC, as it can acquire and reproduce the natural color and flavor of a modified culture medium, thereby not causing major changes to organoleptic properties.[93] The addition of 10% BNC in Chinese meatball showed slight changes in texture but overall similar sensory properties to control.[94] No off-flavors nor changes to texture and mouthfeel was detected in nanocellulose-modified hamburger.[95] Likewise, Sangnark et al confirmed fiber with large particles elicited adverse effects on the grain of bread and reduced overall acceptability of bread, whereas bread with smaller-sized fiber (<0.075) at the same concentration obtained sensory scores remarkably close to the control bread containing 100% wheat flour.[96] This further confirms the promising potential of nanocellulose as a fiber fortificant in food, although additional studies may be required to determine the limit of possible nanocellulose addition before it begin to cause undesirable sensory changes.

Overall, the application of nanocellulose in food may not only provide beneficial effects from the addition of nanocellulose itself, but also on the organoleptic and nutrient quality of food, thus producing a palatable and healthy food that supports the betterment of metabolic syndrome.

CONCLUSION

Functional food is seen as a favorable option to answer the need of health-stimulating therapies with minimum adverse effects. The development of food technology offers a new type of functional fiber which is nanocellulose. Nanocellulose is the nanoscale counterpart of cellulose, demonstrating improved physicochemical properties including larger specific surface area, higher water-holding capacity, higher swelling capacity, biodegradability, steric barrier properties, low density, and gelling properties. It also boasts various functional activities in the gut, such as impaired lipid and carbohydrate digestion and enhanced gut microflora as well as increased short-chain fatty acid production. These activities may translate to hypocholesterolemic properties, hypoglycemic activities, and prebiotic effect that are beneficial for the treatment of metabolic syndrome. In addition, nanocellulose can improve the quality of food without causing unwanted organoleptic changes with less risk of causing gastrointestinal distress, making it suitable for the production of functional food. Evidently, there are still many gaps to explore considering the currently limited studies, especially from animal and human studies, that opens the opportunity for urgently needed studies to confirm the positive results from *in vitro* studies as well as to detect possible adverse effects that may arise from applications in biological systems. As the interest of nanocellulose for food and health continue to rise, we can look forward to more findings from further research in the upcoming years and possibly even actual applications of nanocellulose for health management.

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The Influence of *Aia Tempayang* on Interleukin-2 (IL2) Levels on Female *Rattus Norvegicus Sprague Dawley* Strains in Breast Cancer Prevention

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ABSTRACT

Background: Breast cancer is a condition in which the cells in the breast tissue grow rapidly and uncontrollably. Risk factors for cancer are those caused by carcinogenic factors, behavior, and diet. Breast cancer causes a decrease in immunity. It can be prevented by consuming high-antioxidant foods such as Aia Tempayang. Aia Tempayang is made from caesalpinia sappan l., scaphium scaphigerum/seeds and ocimum basilicum seeds, each of which contains an antioxidant compound that prevents cancer.

Objectives: To analyze the effect of Aia Tempayang on the decline of interleukin-2 (il-2) as a deterrent to breast cancer.

Materials and Methods: Female Sprague Dawley was 35 days old n= 30, and divided into five groups: normal control group (K1); control groups induced by DMBA without intervention (K2); treatment group induced by DMBA + caesalpinia sappan l. 0.072 g + scaphium scaphigerum 0.012 g + basilicum seeds 0.045 g (X1); treatment group induced by DMBA + caesalpinia sappan l 0.144 g + scaphium scaphigerum 0.024 g + basilicum seeds 0.09 g (X2); and treatment group induced by DMBA + caesalpinia sappan l 0.288 g + scaphium scaphigerum 0.048 g + basilicum seeds 0.18 g (X3). After 35 days of intervention, serum IL-2 was analyzed using ELISA method. Data analysis used Paired-T Test, One Way ANOVA, and Post-Hoc Bonferroni follow-up test.

Results: There was a significant difference in serum IL-2 (p=0,000) between supplementation groups found after intervention.The X1, X2, and X3 groups showed decreased of IL-2 to the K2 group without intervention.

Conclusion: Aia tempayang was effective in reducing interleukin-2 levels in the group of mice induced by DMBA for 35 days with doses of X1, X2 and X3. The dose closest to the normal group is the intervention group X3 with a dose of Caesalpinia sappan l 0.288 g + Scaphium scaphigerum 0.048 g + Basilicum seed 0.18 g.

Keywords : breast cancer; interleukin-2; caesalpinia sappan l; scaphium scaphigerum; basilicum seeds.

BACKGROUND

Breast cancer is the most common cancer in women and is the most common cause of death by cancer after lung cancer, with a percentage of 12%. There are 3.5 million women with breast cancer.[1] It is a condition in which cells in breast tissue grow rapidly and do not grow controlled in breast tissue.[2] The emergence of new cells is caused by uncontrolled cell division and is followed by invasion and metastasis of cells into other tissues and organs of the body. Many risk factors cause breast cancer, that are carcinogens, behavior, and food. Carcinogens are cancer-inducing substances that interfere with metabolic processes.[3] Cancer decrease the patient's immunity. Immunity is a reaction in the body against foreign substances that enter the body molecularly or cellularly.[4] Cells involved in the immune system in the body are T cells produced by the thymus and B cells produced in the bone marrow. Cancer cells in the body are responded to

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by the innate and adaptive immune systems, which involve macrophages and T helper (Th) cells. Helper T cells can produce IL-2 cytokines, which are regulators of the growth and differentiation of lymphocyte cells. Macrophages and IL-2 are two components of the immune system.[5] Interleukin-2 (IL-2) produced by T helper cells is a growth factor for all subpopulations of T lymphocyte cells and is responsible for the clonal expansion of T lymphocytes after T lymphocytes recognize antigens.[6]

There have been various types of treatment for breast cancer, but some of these treatments have certain side effects. Meanwhile, several studies have shown that some cancer sufferers use herbal medicine as an alternative treatment. Herbal medicine is used as an immunomodulator and is recognized as the most frequently used alternative treatment for cancer. Immunomodulators are substances that can help improve immune system function.[7]

Herbal medicinal plants have antioxidant active substances that function to boost the immune system and ward off radicals.[8] One of the uses of herbal drinks as an alternative treatment is *Aia Tempayang*, which is a traditional drink typical of West Sumatra that is brewed. The ingredients used are derived from several ingredients, Caesalpinna Sappan, *Scaphium Scaphigerum*, and *Basillicum Seeds*. Each ingredient contains compounds that prevent cancer. Caesalpinia Sappan contains bioactive flavonoids and phenols that play a role as antioxidants, and the main substance contained in sappan wood is brazilin, which acts as an anti-inflammatory, anti-bacterial, anti-diarrhea, and anti-cancer agent.[9] Brazilin substances that can modulate the immune system, especially T cell activity. Thus, it can increase cell activity and suppress the decrease in interleukin-2.[10]

Schapium Affine contains alkaloids, flavonoids, glycosides, tannins, and saponins that are useful for treating diseases such as intestinal infections, fevers, inflammation, asthma, and pharyngitis.[11] The content of methanol and ethanol extracts from *Scaphium Affine* is known to have antioxidant activity that can effectively reduce free radical activity. *Basil Seeds* are one of the ingredients used in steeping. The content contained is antioxidants and contains essential oils as the main component and other components such as tannins, cardiac glycosides, flavonoids, and other phenolic compounds and saponins. Basil essential oil has been shown to have immunomodulatory, hyperglycemic, hypolipidemic, anti-inflammatory, and anti-microbial effects.[9] This research on *Aia Tempayang* has never been done on experimental animals or humans, so the researchers wanted to prove the effect of giving Aia tampayang to decrease interleukin-2 levels.

MATERIALS AND METHODS

The research was carried out from April to May 2021. The ingredients for Aia Tempayang were obtained from the traditional market of the city of Solok, West Sumatra. Intervention research on experimental animals at the Nutrition Laboratory of the Inter – University Center of Food and Nutrition Studies (PSPGPAU), Gajah Mada University,Yogyakarta. Animal Laboratory for 42 days, from acclimatizasation to blood collection for the post test. Research on experimental animals has been approved by the Medical/Health Bioethics Commission, Faculty of Medicine, Sultan Agung Islamic University, Semarang, with the Ethical Clearance number No.28/II/2021/Commission on Bioethics.

Process of Making an *Aia Tempayang*

Aia Tempayang consists of *Caesalpinia Sappan*, *Scaphium Scaphigerum*, and *Basilicum Seeds*. All these items were stored with aluminum foil to prevent physical damage. A brewing process involves mixing all the ingredients and then brewing them at 70^0C temperatures for 20 minutes, then filtering the entire material.

Experimental Animals

In this experimental study, randomized post-test only design with a control group (n=30) on female white wistar rats was used. The subjects of this study were female *Rattus norvegicus* Sprague Dawley (SD) rats that were induced by DMBA at the same time as the intervention. DMBA induction is induced through a subcutaneous areola mouse area twice a week for 5 weeks with dose 20 mg/kgBB. The rats used were female rats aged 35 days with a body weight 150-300 grams, healthy, and active.

The rats are divided randomly into five groups (six female rats per group): normal control group (K1); control groups induced by DMBA without treatment (K2); treatment group induced by DMBA + caesalpinia sappan l 0.072 g + scaphium scaphigerum 0.012 g + basilicum seeds 0.045 g (X1); treatment group induced by DMBA + caesalpinia sappan l 0.144 g + scaphium scaphigerum 0.024 g + basilicum seeds 0.09 g (X2); and treatment group induced by DMBA + caesalpinia sappan l 0.288 g + scaphium scaphigerum 0.048 g + basilicum seeds 0.18 g (X3). The rats are groubed with 27^0C thermometers, 70% higrometers, and AC 17^0c, and the condition of the mouse is 12 dark hours and 12 bright hours. All rats are grouped in the mammary glands to detect abnormal mass development after five weeks of intervention.

The standard feed used is AD II standard feed, composed of 15% crude protein, 3–7% crude fat, 12% water content, 6% crude fiber, 7% ash, 0.9–1.1% calcium, and 0.6-0 phosphorus. 9%). Rat body weight measurements were carried out once every 7 days using a digital animal scale. Examination of interleukin-2 levels was carried out using the ELISA method at the end of the study.

Statistical Analysis

The data used was the primary data of weight measurement and interleukin-2 level examinations, which is a comparison between the healthy control group and intervention group. Weight measurement data was recorded at the beginning of the study and then recorded every week. *SPSS* was used to analysis data.

The data were tested for normality using the Shapiro-Wilk test. The first statistical analysis determined the differences in weight data pre-test and post-test. The average weight of the experimental animals was normally distributed, with the Paired t-test and *One - Way* Anova used to examine changes and differences in the group, and the Kruskal-Wallis test to differences in body weight changes between experimental groups of animals. The second statistical analysis is the post-test carried out on interleukin-2 levels data, which proved the IL-2 Level was normally distributed. Furthemore, the *One-Way* Anova and Bonferroni Post-Hoc tests were used to examine the difference in the intervention effects in the group.

RESULTS

The body weight characteristics of the experimental animals during the acclimatization period ranged from 160–188 g. No experimental animals dropped out during the study. The intervention of Aia Tempayang for 35 days resulted in an increase in the body weight of rats. The results of statistical tests on changes in body weight of experimental animals before and after administration of Aia Tempayang (Table 1) showed an increase in body weight of experimental animals before and after DMBA induction and Aia Tempayang intervention were significantly different in groups (p = 0.000).

Table 1. Test Animal Body Weight Value (g) Before and After Intervention Aia Tempayang

Group	n	Before (Mean±SD)	After (Mean±SD)	*p*	Δ Median (min-Max)
K_1	6	175,83±4,579[a]	216,00±4,858[a]	0,000	40,50(39-41)[b]
K_2	6	178,50±3,728[a]	169,67±3,266[a]	0,000	-9,00(-10- -8)[b]
X_1	6	181,00±4,775[a]	210,33±4,546[a]	0,000	29,50(28-30)[b]
X_2	6	182,67±3,559[a]	222,50 ±	0,000	40,00(38-41)[b]
X_3	6	181,17±5,037[a]	3,450[a]	0,000	40,50(39-41)[b]
			221,33 ± 4,412[a]		P = 0.000[b]

p= Paired T-Test; [a]= One Way ANOVA; [b]= Kruskal-Wallis

The results of the paired t-test in the table above show that there is a significant difference in the average body weight between groups before and after the 35-day aia tempayang intervention in each group (p = 0.000). Based on the Kruskal-Wallis test, it showed that there was a significant difference in weight change between groups (p<0.05).The results of the One-Way ANOVA test showed that there was a significant difference in body weight at the end of the intervention and the DMBA induction on weight changes between groups (p = 0.000). evidenced by p-value < 0.05. Descriptively, the lowest percentage increase in body weight of experimental animals was shown by group K_2, which were DMBA-induced rats without Aia tempayang intervention. The provision of the Aia Tempayang intervention was higher in weight gain in the treatment group and was equivalent to the increase in body weight in the healthy control group who received standard feed.

Table 2. Interleukin-2 Levels in Experimental Animals (pg/dl)

Group	n	Interleukin-2 Levels	*p*
K_1	6	0,226 ± 0,035[a]	0,000
K_2	6	1,317 ± 0,213[a]	
X_1	6	0,799 ± 0,032[a]	
X_2	6	0,425 ± 0,033[a]	
X_3	6	0,290 ± 0,023[a]	

p=One Way Anova, [a]=Post-Hoc Bonferroni

One-way ANOVA statistical test results (Table 2) showed interleukin-2 levels were significantly different in the five groups (p = 0.000). The results of Bonferroni's Post-Hoc statistical test showed an increase in interleukin-2 levels in the K2 (1,317 ± 0,213) group compared to the X1, X2, and X3 intervention groups, whose results were close to those of the normal group (0,226 ± 0,035). This proves that the intervention dose of aia tempayang in groups X1, X2, and X3 has the ability to reduce levels of interleukin-2 in rats with breast cancer. There was no difference in group X3 (p = 0.032) compared to K1. This indicates that steeping Aia tempayang with a dose of *caesalpina sappan* = 0.288 g, *scaphium scaphigerum* = 0.048 g, and *basillicum seeds* = 0.18 g is the best dose to reduce interleukin-2 levels in breast cancer rats. The larger of the dose, the lower the interleukin-2.

DISCUSSION

The weight gain of experimental animals in the intervention group of rats was significantly different compared to the sick rat group. The weight loss that occurred in the K2 group was due to the carcinogenic DMBA, which was given continuously in the absence of received antioxidants.[12] Compared to mice that were not given DMBA, group K1. Weight loss is the result of a nutritional disorder known as cachexia syndrome. In cancer patients, cachexia ranges from 40–80% and causes death in 30–50% of cancer patients.[13] Cachexia occurs due to various factors, that is inadequate food intake, metabolic disorders, and specific humoral and inflammatory responses.[14]

Inteleukin-2 is a cytokine that plays a role in regulating the immune response. It functions as a mitogen for T cells, which potentially increases the proliferation and function of T cells, B cells, and NK cells, improves antigen formation, and increases the production and release of other cytokines. In a stable or normal body state, the amount of IL-2 is reduced by Treg cells, so the amount is quite low. The reduction in the amount of IL-2 by Tregs is in line with the function of Tregs, namely suppressing the immune response, so that there is no excessive immune response, such as autoimmune.[15] The group of rats induced by DMBA and the intervention of Aia tempayang steeped decreased the amount of IL-2 due to a decrease in the number of cancer cells due to apoptosis, so that the signals for the formation of IL-2 were reduced and relatively close to the amount in normal mice. The development and activity of T cells can be stimulated by the addition of an immunomodulator.

Aia Tempayang is a traditional drink made from several food ingredients, it is made from *caesalpina sappan*, *basillicum seeds*, and *scaphium scaphigerum*. Where the secondary metabolite content in each ingredient contains antioxidants and is in the form of chemical compounds, that is flavonoid compounds.[16] This compound can suppress the decrease in the number of leukocytes and has the potential as an anti-inflammatory. One of the community's efforts to suppress the high prevalence of breast cancer is to increase the consumption of antioxidant-rich foods. The flavonoids in the wrought Aia Tempayang can used on interferon γ produced by T cells, which will stimulate phagocytic cells and increase the secretion of IL-2.

In the study of Swarnalatha (2014), it was also stated that flavonoids can affect inflammation and lymphocyte proliferation. In relation to breast cancer, some tumor cells are presented by dendritic cells to induce T cells to proliferate and secrete large amounts of IL-2 and IFN-. Interleukin-2 is found in high amounts in the body tissues of breast cancer patients, especially at the tumor site.[17] This causes an increase in the number of Th2 and Treg cells, which inhibit the immune system's anti-tumor response to cancer. Administration of IL-2 as immunotherapy in cancer patients has also been shown to increase the number of circulating Treg cells, thereby causing a very strong suppression of the immune system.[18] As evidenced by the absence of nodules in the breasts of rats, flavonoids showed selective ability in terms of killing cancer cells and inhibiting cancer angogenesis and cancer metastasis.[19]

Antioxidants have an important role in protecting the body from free radical attack. Antioxidants such as flavanoids, vitamin C, which are found in brewed aia tempayang can boost the immune system to fight and protect the body against cancer and infections.[20] Through this mechanism, the effectiveness of all bioactive compounds and nutrients that act as antioxidants, anti-inflammatory, and anti-cancer agents in Aia Tempayang is enhanced. After 35 days of treatment, the mean decrease in interleukin-2 levels was higher at dose III and was equivalent to that of the healthy control group.

This study had limitations, that the long-term effect of steeping aia tempayang on the prevention of breast cancer is unknown, and it is not known what ingredients are the most effective in the active components of aia tempayang steeping.

CONCLUSIONS

The aia tempayang intervention was able to reduce interleukin-2 levels in the intervention group at a dose of 0.288 g of *caesalpina sappan*, 0.048 g of schapium scaphigerum, and 0.18 g of basillicum seeds and was significantly different compared to the group of rats induced by DMBA without intervention.

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Effects of Dietary Interventions on Gut Microbiome in Overweight or Obese Adults: A Systematic Review of Randomized Controlled Trials

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ABSTRACT

Background: It has been shown that gut microbiota dysbiosis may induce intestinal permeability, and systemic inflammation, leading to metabolic dysregulation. Furthermore, it has been implicated in the etiology of obesity. Dietary intake is known to affect the gut microbiota. These RCTs suggested that different dietary interventions may exhibit different effects on the composition of gut microbiota in overweight or obese individuals.

Objectives: This systematic review aimed to determine the effect of dietary intervention on the gut microbiota profiles in overweight or obese adults. The primary outcome of this systematic review is alpha-beta diversity and its changes at the species level.

Materials and Methods: This systematic review followed the PRISMA guidelines and was registered in the PROSPERO database with registration number CRD42022298891. A systematic search was conducted through the databases PubMed, MEDLINE, CINAHL, and Scopus literature using the terms: "gut microbiota","microbiome","overweight","obesity", "insulin sensitivity","insulin resistance","blood glucose","randomized controlled trial". After screening abstracts and full texts, 18 articles were extracted by two authors.

Results: Among the 18 RCT studies, dietary intervention gave an impact on gut microbiota alpha diversity changes in four studies. However, 7 studies showed no significant changes or differences compared to the placebo group. Beta diversity analysis was reported in 7 among 11 studies that performed alpha diversity analysis. Significant changes were found in food nutrients group (fiber supplementation) studies conducted over 8-12 weeks period. Seven more studies did not report any analysis of variance in either alpha or beta diversity. Changes in the composition of gut microbiota could be observed in dietary pattern interventions and resulting in improved metabolic status, except in the fried meat group diet. Interventions with food groups, food nutrients, and probiotics did not change the composition of gut microbiota.

Conclusion: The effects of dietary interventions on alpha-beta diversity are inconsistent, but rather showed more consistent effects on the changes in microbiota composition, especially in dietary pattern interventions.

Keywords: dietary interventions; gut microbiota; obesity; insulin resistance; randomized controlled trial

BACKGROUND

The prevalence of obesity worldwide has sharply increased according to the World Health Organization in 2016 [1], indicating that 39% of adult population are overweight and more than 13% of them are obese [1]. Obesity is characterized by increase in body-fat mass [2] and low-grade inflammation[3] which may determine chronic diseases such as type 2 diabetes mellitus, stroke, coronary heart disease, several types of cancer, and recently, increasing in mortality caused by COVID-19 infection [4,5].

Obesity is caused by a long-term energy surplus, can be influenced by genetic, neurobiology, and environmental factors [6,7]. Recently, the gut microbiota has been implicated in the etiology of

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obesity [8]. The gut microbiota contains genes that encode thousands of microbial enzymes and metabolites [9] that interact with human as the host to play a role in the immune system and metabolic regulation [10]. Furthermore, the gut microbiota has been shown to regulate glucose homeostasis and lipid metabolism. In addition to that, the gut microbiota metabolites such as short-chain fatty acid are also known to regulate satiety by stimulating the production of hormone peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) via the gut-brain axis [11–14].

Dietary intake is known to affect the gut microbiota [15]. Cumulative evidence has shown that western diet rich in saturated fat and low in fibre decreases the composition of gut microbiota towards dysbiosis. It has been shown that gut microbiota dysbiosis may induce intestinal permeability, fat colonization, and systemic inflammation, leading to metabolic dysregulation [16]. An intervention study with 40% of the total energy consisted of high-fat diet (HFD) showed changes in the composition of gut microbiota in which associated with an increase in Alistipes, a decrease in Faecalibactreium and concentration of short-chain fatty acids (SCFA), as well as an increase in arachidonic acid, lipopolysaccharide biosynthesis and inflammatory cytokines (CRP) [17].

On the other hand, the dietary pattern of Mediterranean diet, which is rich in vegetables, fruit, nuts, whole grains, fish, and olive oil, showed a decrease in the phylum Firmicutes and was followed by the level of *Ruminococcus gnavus* species which has potential for pro-inflammatory conditions [18]. Supported by an acute intervention study of the Mediterranean diet for four days, it showed changes in the abundance of fibre-fermenting bacteria, such as *Lachnospiraceae* and *Butyricicoccus*, which increased significantly and decreased after being given a high-fat diet [19]. Another study of probiotic strain *A. muciniphilla* supplementation was able to increase insulin sensitivity index by 30% compared to placebo [20]. Moreover, an intervention using inulin and resistant starch within 24 hours showed an increasing trend of SCFA production [21].

These randomized controlled trials (RCT) suggested that different dietary interventions may exhibit differences effect in the composition of gut microbiota in overweight or obese individuals. Therefore, we conducted this systematic review to determine the effect of dietary intervention on the gut microbiota profiles in overweight or obese adults. The primary outcome of this systematic review is alpha-beta diversity and its changes at species level.

MATERIALS AND METHODS

This systematic review was carried out by published protocols and refers to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Guideline 2020[22]. This systematic review protocol has been registered in the Prospective Register of Systematic Reviews, PROSPERO (reg.no. CRD42022298891).

Data Source and Collection

A comprehensive literature search was conducted through PubMed, MEDLINE (Medical Literature Analysis and Retrieval System Online), CINAHL (Cumulative Index to Nursing and Allied Health Literature), and Scopus. The main keywords used were as follows: gut microbiota, microbiome, overweight, obesity, insulin sensitivity, insulin resistance, blood glucose, randomized controlled trial. These keywords were combined with Boolean operators (e.g. OR, AND, NOT), and all fields or MeSH (Medical Subject Heading) terms.

The interest of primary outcome was the change in gut microbiota profile seen from alpha diversity, beta diversity, and richness or abundance on specific species levels. The association of specific species with fasting insulin and fasting glucose could also be reported as a secondary outcome.

Study Selection

Eligibility criteria included studies that met the PICOS (Patients/participants, Intervention, Comparison/control group, Outcome, and Study design) in which the study population consisted of 1) individuals with overweight and/or obesity (with or without insulin resistance, metabolic syndrome; 2) adults only (more than 18 years old); 3) the minimum duration of the intervention is 1 month (4 weeks); 4) diet intervention is performed daily; 5) the reported outcome is gut microbiota profile included alpha-diversity, beta diversity and richness/abundance of species level, fasting insulin, fasting glucose; and 6) the experiment is in RCT. Exclusion criteria were consisted of individual with type 1 diabetes, type 2 diabetes, gestational diabetes, NASH, NAFLD, infectious diseases (e.g. HIV, TB, COVID-19), cancer disease, gastrointestinal disease, end-stage renal disease, neurological disease, and studies performed in adolescents or children.

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During the collection process, duplicate studies were manually identified and removed. The articles were then analyzed using two-steps procedure. Initially, the retrieved titles and abstracts were independently analyzed by two authors. Next, the full text of all included articles were subjected to other analysis, then the eligible articles based on the inclusion and exclusion criteria were identified. Disagreements were resolved by consensus-based discussion or by another author's opinion.

Data Extraction and Quality

Assessment

Two authors (TAST and AP) independently extracted data from each study using an extract table template (Ms. Excel). Data extracted included: first author, year of publication, study design, place of study, participants' characteristics (*n*, age, BMI), dietary intervention (whole diet or specific diet), duration of interventions, and outcome measures (change of alpha -beta diversity is derived from any tools such as Shannon or Simpsons Index, Chao-1, UniFrac distance, specific species level of richness or abundance, and associated of specific species with fasting insulin and fasting glucose). Each RCT was assessed for its quality using the National Heart, Lung, and Blood Institute (NHLBI) tool for intervention studies. Four of these items represented fatal flaws if answered "No/Not reported/ Can't determine": (i) randomization (#1), (ii) dropout rate <20% (#7), (iii) valid/reliable outcome measure (#11), (iv) intent-to-treat analysis (#14). Study quality was determined based on the number of fatal flaws: good quality (0 fatal flaws), fair quality (1 fatal flaws), or poor quality (≥ 2 fatal flaws).

Data Synthesis

The findings from the included studies would be presented as a narrative synthesis and illustrated in a table, including the following information; authors, year, subject characteristics, type of intervention, duration, primary outcome, and secondary outcome. Subgroups analysis would be conducted for different types of dietary interventions and based on durations.

RESULTS

Study Selection

The literature search identified 130 studies that have been reported in PubMed (63); Medline (10); CHINAHL (19); Scopus (38), and after excluding the duplicates and non-eligible titles and abstracts, 29 original articles were included. The identification and study selection detail are presented in the PRISMA flow chart (Figure 1).

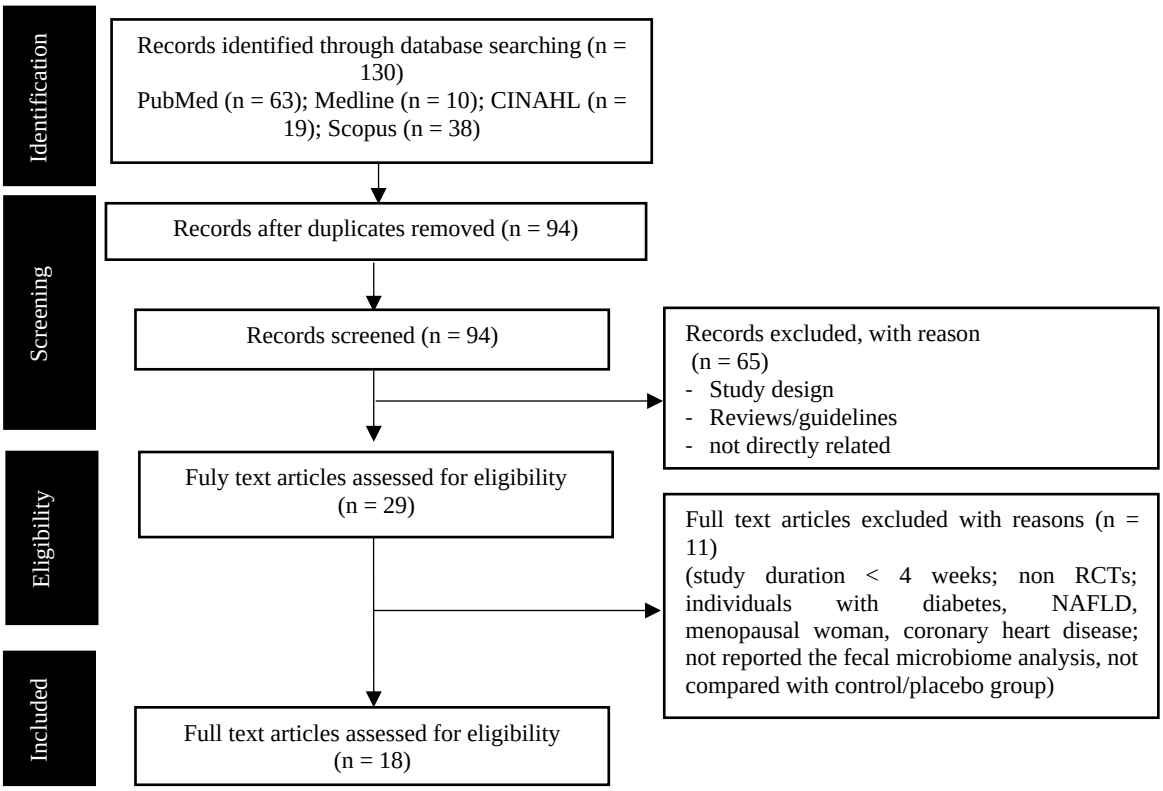

Figure 1. Flowchart Diagram for Study Selection of Systematic Review (based on PRISMA Guideline)

After a review of the full text some studies were excluded: 2 non-RCT studies, 6 ineligible studies according to the inclusion criteria, 1 study without fecal microbiota analysis, 1 study with less than 4 weeks duration, and 1 study without comparison to the control or placebo group. Finally, 18 studies were selected for review.

Study Characteristics

Characteristics of these studies are shown in Table 1, in relation to this review, study design, study location, participants, sample size, age, body composition (e.g. body mass index/BMI), health status, dietary intervention, and duration were also included. In total, 18 RCTs were selected [23,24, 25–32,33–40,] with publication year was between 2013-2021. There were 9 studies enrolled healthy overweight or obese participants, meanwhile the remaining studies were conducted on overweight or obese individual with insulin resistance, metabolic syndrome. Among the 18 RCT studies, 1 was single-blinded, 11 were double-blinded, 3 were open-labelled, and 3 had no report on the study design.

Any dietary interventions in the studies were classified into many sub-categories [41] such as dietary patterns (e.g., Mediterranean diet, high fat diet, vegan diet, multifunctional diet), food groups (e.g., kimchi, the red wine polyphenol, pea fiber, and whole grain), food nutrients (inulin, arabinoxylan oligosaccharides, genistein supplementation) and probiotic supplementation which was compared to the control or placebo group. The duration the intervention vary from a minimum of 4 weeks to 1 year.

Dietary Interventions and Gut Microbiota Diversity (α and -diversity)

Table 2 shows an alpha-beta diversity analysis. Alpha diversity was only measured in 11 [23,24,40,25,27,28,32,35,37–39] out of 18 reviewed studies. The measurement of alpha diversity used the Shannon index, Simpson index, and Chao1. Dietary intervention gave impact in in gut microbiota diversity changes in four studies [37–40]. However, 7 studies [23–25,27,28,32,35] showed no significant changes or differences compared to the control/placebo group. Dietary intervention with a period of more than four weeks showed changes in alpha diversity, both enrichment and decreased diversity. One of them, Vitale et al., 8 weeks intervention of Mediterranean diet showed a significant increase in gut microbiota diversity compared to the Western diet [37]. Similar intervention in obese adults with metabolic syndrome within one year of period by Muralidharan et al. showed no change in alpha diversity compared to baseline[28].

Beta diversity analysis reported in 7 [23–25,27,28,39,42] among 11 studies [23–25,27,28,32,37–40] which performed alpha diversity analysis. Significant changes were found in food nutrients group (fiber supplementation) studies conducted over 8-12 weeks period. Seven more studies [26,29–31,33,34,36] did not report any analysis of variance in either alpha or beta diversity.

Changes in the diversity of the gut microbiota were not only from food intake and food composition, but also from dietary patterns and the environment (such as lighting settings) affecting circadian rhythms which influence the composition of microbiota[43]. It is interesting to consider it during the intervention which was not found in this review.

Dietary Interventions, Bacterial Taxa, and Metabolic Marker Changes

The abundance of bacterial taxa is reported in this study review; 15 studies were analyzed up to species level and 3 studies[25,40,44] were analyzed up to genus levels. Details of the results are reported in Figure 2.

<table>
<tr><th colspan="2">DIETARY PATTERNS*</th><th colspan="2">FOOD GROUPS*</th><th colspan="2">FOOD NUTRIENT*</th><th>PROBIOTIC</th></tr>
<tr><td>Vegan</td><td>↔ Firmicutes/Bacteroidetes

Species:
↑ Faecalibacterium prausnitzii</td><td>All Groups (Fermented and Non Fermented Kimchi)</td><td>↓ Firmicutes/Bacteroidetes</td><td>IPE</td><td>↓ Firmicutes (B. obeum, E. ruminantium)
↑ Bacteroidetes spp (B. uniformis, B. xylanisolvens)</td><td rowspan="4">Changes based on the probiotic given.</td></tr>
<tr><td>Mediteranian Diet</td><td>↑ Bacteroidetes/Firmicutes

Species:
↑ Faecalibacterium prausnitzii
↑ A. muciniphilla</td><td>Redwine</td><td>Species:
↑ Faecalibacterium prausnitzii</td><td>AXOS</td><td>Species:
↑ Faecalibacterium prausnitzii</td></tr>
<tr><td rowspan="2">Fried Meat (High Fat Diet)</td><td rowspan="2">↓ Firmicutes/Bacteroidetes

↑ Dialister, Dorea, and Veilloella as a pathogenic bacterium</td><td colspan="2">Refined vs. Whole Grains</td><td rowspan="2">Genistein Group</td><td rowspan="2">↓Firmicutes/Bacteroidetes

Species:
↑ A. muciniphilla</td></tr>
<tr><td>Whole Grains</td><td>Species:
↑ Faecalibacterium prausnitzii</td></tr>
</table>

Figure 2. Primary Outcome for Gut Microbiome Taxa Following Dietary Interventions

*Grouping of dietary interventions are reffered on the reference (Toi PL, Anothasintawee T, Chaikledkaew U, Briones JR, Reutrakul S, Thakkinstian A. Preventive Role of Diet Interventions and Dietary Factors in Type 2 Diabetes Mellitus: An Umbrella Review. *Nutrients*, 2020;12(9):2722. Published 2020 Sep 6, doi:10.3390/nu12092722

Dietary Pattern Interventions

Mediterranean Diet

There were 3 clinical trials that used Mediterranean diet for the intervention. The main characteristics of this diet is high in fiber, indicated by consumption patterns rich in fruits, vegetables, whole grain foods, and low in saturated fat (e.g. olive oil). A study by Muralidharan et al. reported that subjects with metabolic syndrome experienced a decrease in body weight and an increase in the ration of Bacteroidetes/Firmicutes after one year of intervention with the combination of Mediterraniean diets, calorie restriction, and physical activity, compared to the control group[28]. This study is in line with the study by Remely et al. revealing that there was an increase in the abundance of Bacteroidetes after a weight loss in obese individuals [45]. In taxa composition, there were differences in abundance between the two intervention and control groups. Reduction was observed in the genus of Firmicutes, *Butyricicoccus, Eubacterium halii,* and *Ruminiclostridium 5*, meanwhile in the intervention group, the genus of Coprobacter was seen increased, as well as the species of *Ruminococcaceae NK4A214* and *Lachnospiraceae NK4A*136 from the family of Lachnospiraceae [28].

In other intervention studies using Mediterranean diet, Tagliamonte et al. and Vitale et al. found that the healthy-obese group under treatment for eight weeks showed consistent results in the increasing amount of *Akkermansia muciniphilla* [26,37], which are markers for healthy gut bacteria from the phylum of Verrucomicrobia [46].

Three interventions with Mediterranean diets demonstrated favorable metabolic changes to lower glucose and insulin levels [26,28,37]. There was also an increase in postprandial plasma butyric acid after intervention [37], considering that butyric acid provides health benefits in several studies [47]. On the other side, a study by Muralidharan et al. did not report SCFA metabolome analysis. It only included an analysis on related producer bacteria (*Lachnospira* and *Lachnospiraceae NK4A136*) which number was increased in both intervention and control groups [28].

Multifunctional Diet (MFD)

Marungruang et al. mentioned that a multifunctional dietary intervention (MFD) diet enriched with natural antioxidant food and omega-3 supplementation also showed a significant increase in the ratio of Prevotella/Bacteroides, including the species of *Prevotella copri*. Metabolic improvement which marked by lowered diastolic blood pressure, total cholesterol, LDL-cholesterol, and triglycerides. Following those improvements, weight loss was observed after eight weeks compared to the baseline. However, there was no statistical report of taxa abundance correlated between them [23].

Vegan Diet

In study by Kahleova et al., the intervention group was given low fat-vegan diet and vitamin B12 supplementation. Participants were requested to consistently eat a diet rich in vegetables, grains, legumes, and fruit, while avoiding animal products and added oils. The results showed a non-significant increase in the abundance of Bacteroidetes. However, reduction in body weight and improved insulin sensitivity were observed, although it had negative correlation with *Bacteroidetes fragilis* species. SCFA-producing bacteria, the species *Faecalibacterium prautnizii* (butyrate producer) showed increasing in abundance in the low-fat vegan diet group. Although there was a decrease in *Bacteroidetes fragilis* in both intervention groups, but in the vegan diet group was slightly higher compared to the control group [35].

Low Fiber Diet / High Fat Diet

A study by Gao Jian et al., using fried meat as the diet[40], showed contradictive results to those in Mediterranean diet. This finding strengthened the evidence of gut microbiota changes following the high fiber diet especially in individuals consuming Mediterranean diet [26,28,37]. An increase in the abundance of *Dialister* and *Veilloella* from the Firmicutes group occurred in the fried meat group. Deep-frying was used as cooking method to fry the meat during 4 weeks and resulted in a drop of SCFA producing bacteria (*Lachnospiraceae* and *Flavonifractor*). This proved that the result of fried meat intervention was identical to the high-fat diet, in which a significant decrease of butyric acid, valeric acid, and an increase in lipopolysaccharide were observed. Insulin levels was also amplified by an increase in muscle insulin resistance index (MIRI) and several inflammatory cytokines.TNF-α, IL-10, and IL-1β) [40].

Fava et al. reported that total bacteria was decreased after subjects were given high-fat diet. Interestingly, reduction in fat intake accompanied by increasing in carbohydrate intake (thigh carbohydrate

(HC)/high glycemic index (HGI) group) exhibited an increase in *Bacteroides* and *Bifidobacterium spp* that independently increase energy regulation homeostasis. Lowered fasting blood glucose and plasma insulin levels were also observed in the groups taking high carbohydrate (HC)/low glycemic index (LGI) and HC/HGI. Regarding the fecal SCFA, the HS group showed a significant increase in butyric and propionic acids. Fecal SCFA and plasma SCFA levels may provide different interpretations of host metabolism which should be investigated further.

Food Groups

Whole Grain vs Refined Grain

Roager et al. investigated two dietary intervention studies, in which each duration was 8 weeks. The subjects were given either whole-grain diet or refined-grain diet. A total of 60 adults at risk of developing metabolic syndrome participated in these randomized controlled cross-over design studies. It was found that the whole-grain dietary intervention did not significantly impact the changes of bacterial species composition and diversity in the gut microbiota (even after FDR correction for multiple tests was performed). However, among several species, *Faecalibacterium prautsnitzii* and *Prevotella copri* responded with an increase in abundance after being given whole-grain diet, while decreased immediately after consumption of refined-grain diet. Decreasing in body weight, fat-free mass, and inflammatory biomarkers (IL-6) after adjusting for weight loss was observed[32].

Kimchi

Kimchi is a traditional food from South Korean. It is a fermented food with main ingredients are Chinese cabbage, garlic, red pepper, green onion, and ginger. Han et al. compared the difference of giving fresh kimchi vs fermented kimchi to overweight/obese individuals during 8 weeks. The result showed that the group receiving fermented kimchi had greater proportion of bacterial strains than those receiving fresh kimchi. Some changes observed towards the ratio of bacteria after fermented kimchi administration were as follow: 1) the number of Firmicutes and Bacteroidetes were dropped; 2) *Bacteroides* and Prevotella were escalated; and 3) Blautia was decreased. On the other side, in the group consuming fresh kimchi, there was a slight reduction in fasting glucose and insulin levels in comparison to the baseline. This result was not found in the group consuming fermented kimchi[33].

Red wine

A cross-over RCT study with red wine polyphenol supplementation in individuals with metabolic syndrome by Moreno et al. reported that there was an increase in *Bifidobacterium bifidum* species after 30 days of red wine supplementation. At baseline, microbiota analysis showed low abundance of several species such as *Bifidobacterium, Eggerthella lenta, Prevotella, Blautia coccoides–Eubacterium rectale group, Lactobacillus, Faecalibacterium prausnitzii and Roseburia.* A consistent increase in *Faecalibacterium prausnitzii* from the phylum Firmicutes in both intervention and control groups, although there was no significant difference between the two groups. This might happen because red wine polyphenols have prebiotic capacity that presents positive effect towards fasting blood glucose levels, LPS concentrations, and CRP levels in Mets individuals.[36].

Food nutrients

Fiber

The composition, diversity, and abundance of gut microbiota are largely influenced by fiber consumption. Fiber provides a number of metabolic substrates that are used for fermentation reactions carried out by the gut microbiota [48]. In this review, there are three studies with fiber supplementation that consistently show increasing in the Firmicutes phylum [24,30,38].

An intervention study by Chambers et al. that supplemented the subjects with 20 g/day of inulin-propinate ester (IPE), resulted in a rise of Firmicutes phylum, with species bacteria from *B. obeum, E. ruminantium,* meanwhile in the inulin group the subject experienced an increase in *B. obeum, B. luti, B. faecis, R. faecis, Oscillibacter spp*. Similarly, supplementation of IPE or inulin only significantly improved insulin sensitivity (HOMA index, Matsuda index) when compared to cellulose administration [38].

A crossover-RCT study about supplementation of arabinoxylan (AXOS) and PUFA on central obese individuals with metabolic syndrome by Kjolbaek at al., resulted in a drop of Bacteroidetes phylum abundance and a rise in the abundance of Actinobacteria and Bifidobacteria phylum. It also showed an abundance increasing of butyrate-producing species from Firmicutes phylum and Clostridales order such as *Eubacterium rectale, Faecalibacterium prausnitzii, Blautia wexlerae, Dorea longicatena, Eubacterium hallii, Blautia luti, Ruminococcus obeum, Fusicatenibacter saccharivorans*. Unfortunately, there was no washout period between supplementation of AXOS and PUFA. On the other hand, both AXOS and PUFA

supplementation did not result in the changes of metabolic parameters. [24]. Meanwhile, there was no change observed in the composition of gut microbiota after PUFA supplementation.

Supplementation of 15 g/d yellow pea fiber for 12 weeks in a study conducted by Lambert et al. showed a non-significant increase in total bacteria. However, there was a trend of increasing gut microbiota abundance, which was followed by an increase in the abundance of *Clostridium leptum, Clostridium cluster I,* and *Roseburia spp* when compared to the placebo group. This supplementation has a slightly significant metabolic effect to improve body fat profile and glucose tolerance [30].

Genistein

Genistein is an isoflavone compound found in foods, especially soybeans. Genistein is able to modulate the composition of the gut microbiota and reduce metabolic endotoxemia (LPS) and is able to increase insulin sensitivity as indicated by the HOMA index or Matsuda index. A study by Guevara et al. showed a decrease in the Firmicutes/Bacteroidetes ratio, an increase in the Verrucomicrobia phylum, and abundance increasing A. municiphilla, Ruminococcos bromii, and B. uniformis after 50 mg/day of genistein supplementation for 2 months in comparison to the placebo group[39].

Probiotic Supplementation

There are 4 studies of probiotic supplementation in this review; all of them reported changes in the composition of gut microbiota and metabolic markers after supplementation[25,27,29,34] although there was no change in total composition of bacteria.[27]. The metabolic markers observed were reduced insulin levels [25], fasting blood glucose [29], improved insulin sensitivity [34], and improvement of cell-mediated incretin function occurred in *L.reuteri* probiotic supplementation..

A study by Teronio et al. using Lactobacillus spp supplementation with dosage of 9log10 cfu/capsule and omega-3 in individuals with metabolic syndrome resulted in increasing of *Verrucomicrobia* phylum with specific genus is Akkermansia. Significant differences in changes of the metabolic markers between both groups (prebiotic and omega-3) were also observed. A trend of reduced insulin levels, inflammatory cytokines (IL-6), and soluble vascular cell adhesion molecule 1 (sVCAM) which were closely associated with cardiovascular disease risk was also seen [25].

Similar results in a study by Depomnier et al. showed an inclined number of *A. muciniphila* after administration of living *A. muciniphila* or pasteurized *A. muciniphila* [34]. Pasteurized *A. muciniphila* markedly and significantly improved insulin sensitivity index by about 30% as compared to the placebo group.

A double blind RCT probiotic supplementation by Rajkumar et al. (group 1: placebo, group 2: VSL3, group 3 = omega 3, group 4 =VSL3 and omega 3 capsules) showed a significant increase in total bacteria aerobes, *Lactobacillus, Bifidobacteria* and *Streptococcus* in groups 2 and 4. Interestingly, the total bacteria Bacteroides, Coliforms, and *Escherichia coli* was changed in group 4. It was only group 3 that showed no changes [29]

Quality of Included Studies

Table 3. Quality Assessment of Studies

Author	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	Quality
Fava et al. 2013	Y	Y	NR	N	CD	N	N	Y	Y	Y	Y	Y	N	N	Poor
Rajkumar et al. 2014	Y	NR	N	Y	Y	Y	Y	Y	CD	Y	Y	Y	Y	Y	Fair
Han et al. 2015	Y	NR	NR	NR	NR	Y	Y	Y	Y	Y	Y	Y	N	N	Fair
Simon et al. 2015	Y	N	N	Y	N	Y	Y	Y	Y	Y	Y	Y	N	Y	Fair
Moreno et al. 2016	Y	NR	NR	NR	NR	N	Y	Y	Y	Y	Y	NR	NR	Y	Fair
Lambert et al. 2017	Y	Y	Y	Y	Y	Y	Y	Y	Y	NA	Y	Y	Y	N	Fair
Marungruang et al. 2017	Y	Y	Y	NR	NR	Y	Y	Y	NR	NA	Y	Y	Y	N	Fair
Roager et al. 2017	Y	N	N	N	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Poor
Chambers et al. 2019	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	NA	Y	Good
Depomnier et al. 2019	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	Fair
Kjolbaek et al. 2019	Y	Y	N	N	N	Y	Y	Y	Y	Y	Y	Y	Y	N	Poor
Guevara et al. 2020	Y	CD	N	Y	Y	Y	Y	Y	NR	Y	Y	NR	Y	Y	Fair
Teronio et al. 2019	Y	N	NR	NR	NR	Y	Y	Y	Y	Y	Y	Y	N	Y	Fair

Author	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	Quality
Kahleova et al. 2020	Y	Y	N	N	Y	Y	N	N	Y	Y	Y	Y	N	N	Poor
Vitale et al. 2020	Y	Y	Y	Y	NR	Y	Y	Y	Y	Y	Y	Y	N	Y	Good
Gao Jian et al. 2021	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Good
Muralidharan et al. 2021	Y	Y	Y	Y	Y	Y	N	N	Y	Y	Y	Y	N	N	Poor
Tagliamonte et al. 2021	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Fair

Of the 18 included studies in this review (Table 3), the methodogical qualities of three studies were stated to be good, ten were classified as fair, and five were placed as poor. Related the prominent flaws, four studies did not use randomized controlled design; two studies presented droupout rates above 20%, and eight studies did not perform intention to treat analysis.

DISCUSSION

In general, it is difficult to draw conclusions about the effect of dietary intervention in this regard. Metabolic health status, type of dietary intervention, duration of intervention, study design, and sample size may lead to different outcomes among studies. Although there are differences in study results, most of the studies showed association between taxa-specific changes after dietary intervention was given and the metabolic health parameters such as glucose metabolism, fat, insulin sensitivity, inflammation, and some SCFA metabolites due to changes in taxa composition.

Dietary intervention is an important external factor because it influenced the composition and abundance of the gut microbiota. The nutrients contained in the foods are used for survival of the microbes in the digestive tract [49]. Beneficial bacteria is associated with high-fiber intake in either dietary pattern intervention (Mediterranian diet, MFD diet, vegan diet), food groups (kimchi), or food nutrients (inulin, axos supplementation). Similar effects were confirmed by the systematic review by Wagenaar et al [50].

There is an increase in the genus of Lachnospiraceae NK4A136 from the family of Lachnospiraceae [28], species of *Faecalibacterium prautsnitzii* [24,32,35], *Eubacterium rectale*, *Eubacterium halii* [24], and *Roseburia spp* [30]. Several types of these bacteria are believed to be part of the butyrate-producing taxa [51]. A rising number of *Akkermansia muciniphilla* also occurred after the administration of Mediterranean diet[26,37] and fiber supplementation (AXOS)[24]. *Akkermansia muciniphila* has an important role to maintain intestinal barrier function as a feature of healthy gut profile [46,52]. The number of *Prevotella copri* was inclined after being given whole-grain diet but immediately dropped when the diet was replaced with refined-grains[32].

High-fat and low-fiber consumption pattern such as in typical Western diet can reduce total bacteria, as well as the abundance of the Lachnospiraceae and Flavonifractor families (SCFA-producing bacteria)[33,40], meanwhile the amount of pathogenic bacteria which is closely related to obesity was increased [40,53]. In another study conducted by Mocanu et al., low-fiber diet did not change the composition and diversity of bacteria, but on the other side, changes after fecal microbial transplantation (FMT) was found [54].

High-fiber intake in a whole-diet intervention showed improvements in metabolic parameters such as improved insulin sensitivity [26,28,35,37], diastolic blood pressure, total cholesterol, LDL and triglycerides [23], an inflammatory cytokine (IL-6), reduced glucose levels[32], and an increase in postprandial butyric acid after Mediterranean diet intervention [37].

Some supplements such as IPE and inulin improved insulin sensitivity compared to cellulose administration. On the other hand, AXOS supplementation did not show significant changes in blood pressure, glucose metabolism, and lipids[24]. Similarly, fibre supplementation from yellow pea resulted in a slight improvement on body fat and glucose tolerance [30].

Phytochemicals (polyphenols) are able to provide two effects on the gut microbiota; 1) inhibiting the growth of certain taxa, and 2) increasing the growth of bacteria because they can be metabolized into substrates for the host[55]. Regarding these properties, red wine-polyphenols administration can increase the abundance of *Faecalibacterium prausnitzii* and affect the metabolic health by decreasing fasting blood glucose levels, LPS concentrations and CRP levels[36]. Another type of phytochemicals is genistein, which in some studies were shown a rising number of *Akkermansia muciniphilla* after genistein supplementation and significantly improved the insulin sensitivity by 30% compared to the control group[39].

Faecalibacterium prautsnitzii is butyrate-producing microbiota that consistently presents in high-fiber intake (Mediterranean, Vegan, and AXOS) and in red wine-polyphenol interventions. Dietary fibre is a carbohydrate polymer that cannot be metabolised by amylase[56] and cannot be absorbed in small intestine[57].

Dietary fibre can only be processed by certain species of gut microbiota through an anaerobic fermentation process, with the main product being SCFA (butyrate, propionate, acetate) [48]. Short-chain fatty acid is a metabolite products that play a role in energy regulation processes, immune system, and cell proliferation[58]. Butyrate is present in a greater proportion in the intestinal lumen[58].

Gram-positive butyrate-producing bacteria plays important role in gut health, by carrying out the fermentation process in the colon. The process takes place by obtaining ATP through substrate-level phosphorylation during the breakdown of oxidative-substrate[48]. Then, combination of two molecules of acetyl-CoA to form acetoacetyl-CoA, accompanied by a gradual reduction to butyryl-CoA for the next step in the formation of butyrate [51]. Intestinal mucosa requires butyrate as its main energy and plays a role in the regulation of gene expression, inflammation, differentiation, and apoptosis in host cells. Butyrate is able to reduce the negative effects of lipopolysaccharides and simultaneously increase the regulation of intestinal barrier function by stimulating the production of mucin, as well as activation of G-protein receptors (GPR41 and GPR43) in the large intestine, stimulation of the production of hormone peptide YY (PYY) and glucagon-like peptide. -1 (GLP-1) which affects glucose homeostasis.[11].

High-fat and low-fibre diets cause gut bacteria to use mucin glycans for self-metabolism by inducing the expression of enzyme genes that result in decreased the mucin [57], leading to a risk of leaky gut barrier which is characterised by increased permeation of luminal contents into the mucosa and submucosa layer around immune cells. This condition has potential to cause translocation of lipopolysaccharide compounds (LPS) into the blood circulation[59] which leads to metabolic dysregulation [16]

In the fried-meat intervention group, a drop in butyric and valeric acid, a rise in lipopolysaccharide and insulin levels were occurred due to amplification by an increase in muscle insulin resistance index (MIRI) and several inflammatory cytokines (TNF-α, IL-10, and IL-1β).[40]. In addition, the study reported by Fava et al in the high showed a significant increase in fecal butyrate and propionate. In this perspective, the increase in fecal SCFA in obese individuals is interesting to understand in regard to the intestinal absorption function and/or activity modulation of the composition of gut microbiota present[44]. Moreover, it is probably the choice of plasma SCFA measurement as in the study of Vitale et al can be considered to assess the exact amount of SCFA levels [37].

Probiotic supplementation also provides significant changes in the composition and abundance of gut microbiota, excluding symbiotic supplementation[60] and only omega-3 supplementation without any combination with probiotics[29]. An increase in *Akkermansia muciniphilla* appeared after supplementation with the probiotic Lactobacillus, in the form of living or pasteurized *A. Muciniphilla* [46,52]. Reduced insulin levels[25] and fasting blood glucose[29], improvement in insulin sensitivity[34] and cell-mediated incretin function occurred with probiotic supplementation of *L. reuteri*,[27].

The plausible mechanism that might explain the improvement in metabolic health is the ability of probiotics to repair the intestinal epithelial barrier and reduce intestinal permeability due to previous dietary consumption patterns, leading to inflammation suppression and translocation of endotoxins into the blood vessels[61,62]. Positive correlation in regards to improved insulin sensitivity was reported in relation to bacterial species of A. Muciniphilla [34], *Bacteroides fragilis* [35], *Bacteroides stercoris, Bacteroides caccae, Phascolarctobacterium faecium* [54], *Intestimonas butryriciproducens, Desulfovibrio piger, Coprobacter fastidious s*[24], one OTU (unpublished species name) classified as *Prevotella* genus [44], and also *Lactobacillus reuteriI*. Meanwhile, negative correlation was found in the types of bacteria *Dialister succinatiphilus*, Turcibacter sanguinis, Alloprevotella [24]. In addition, *Faecalibacterium prausnitzii* [28], Eubacterium eligens [37] has negative correlation with blood glucose levels

Akkermansia muciniphilla is one of species that appears consistently for a minimum of eight weeks duration [25,26,37,39] (more details in Table 3). Nine dietary intervention studies were administered to healthy overweight/obese individuals. Seven studies (78%) demonstrated significant improvements in metabolic health, occurring at a minimum duration of four weeks of intervention [23,26,30,35,37,38,40]. Two studies in the obese group with insulin resistance showed improvement in metabolic status beginning at week 6 [29,39]. One study in pre-diabetic individuals did not report any change in metabolic markers [63].

Inconsistent changes were also still visible at long duration of 10-12 weeks [24,25,34,36,54], but starting at 28 weeks up to one year of the duration, it showed significant change results [28,33]. These results possibly indicated that individuals with metabolic syndrome require a long-term diet to achieve significant change. Several other studies suggest the possibility that there is no significant effect of diet on gut microbiota and metabolic health, depending on interpersonal variability in enterotype composition, even if the individuals have been subjected to appropriate extreme diets [64]. Therefore, further mechanisms need to be investigated.

In this review, differences in the diversity and composition of gut microbiota taxa may be influenced by factors such as metabolic health status, duration of intervention and types in the dietary intervention itself. In general, a high-fibre diet and dietary interventions rich in polyphenols (there is prebiotic capacity) have a positive effect on the diversity and composition of beneficial microbes that are beneficial in improving metabolic function. It could be partly associated with increased levels of SCFA through the process of bacterial fermentation[44]. Furthermore, the effectiveness of probiotic administration depends on the specific strain used on the initial composition of the individual gut microbiota. Finally, the limited included studies in this review might also be a major limitation of this systematic review; thus, further investigation in a large well-controlled study is needed to confirm these findings

CONCLUSIONS

In conclusion**,** the effects of dietary interventions on alpha-beta diversity are inconsistent but showed more consistent effects on microbiota composition changes. Meanwhile, interventions with food groups, food nutrients, and probiotic did not change gut microbiota composition. Changes in gut microbiota composition could be observed after modifying the diets, resulting in improved metabolic status. However, this does not include the group receiving dietary interventions with fried meat. Future studies on the effects of cooking methods on the gut microbiota and metabolic health status are interesting to investigate further.

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Table 1. Characteristic of Studies

Author	Design	Location	N (subject)		n, GM was analyzed	age (years)	BMI (kg/m^2)	Health Status	Intervention	Duration
			pre	post						
Dietary pattern										
Fava et al. 2013	SB-RCT	UK	130	88 (HS group = 11, HM/HGI group = 17, HM/LGI gorup = 22, HC/HGI group = 21, HC/LGI group = 17)	88 (HS group = 11, HM/HGI group = 17, HM/LGI gorup = 22, HC/HGI group = 21, HC/LGI group = 17)	54±9,5	28,8±4,9	obese with metabolic syndrome	Following a high saturated fat diet (HS) - high glycemic index (GI) diet (total fat 38%E fat, SFA 18%E, MUFA 12%E, PUFA 6%E, CHO 45%E, GI 64%), after which participants were randomly assigned to one of four experimental diets (HM/HGI: total fat 38%E, SFA 10%E, MUFA 20%E, PUFA 6%E, CHO 45%E, GI 64%; HM/LGI: total fat 38%E, SFA 10%E, MUFA 20%E, PUFA 6%E, CHO 45%E, GI 53%; HC/HGI: total fat 28%E, SFA 10%E, MUFA 11%E, PUFA 6%E, CHO 55%E, GI 64%; HC/LGI: total fat 28%E, SFA 10%E, MUFA 11%E, PUFA 6%E, CHO 55%E, GI 51%)	28 weeks (4 weeks run out HS diet, 24 weeks one of four diet intervention HM/HGI; HM/LGI; HC/HGI; HC/LGI)
Marungruang et al. 2017	RCT	Sweden	52 (multifunctional diet = 25, control diet = 27)	47 (multifunctional diet = 23, control diet = 24)	47 (multifunctional diet = 23, control diet = 24)	50-73	25-33	obese	MFD group were given foods rich in natural antioxidants, omega-3 fatty acids, high-(prebiotic) fiber, low glycemic, blood cholesterol-normalizing ingredients. MFD provided 2 g stanol/d for women and 2,7 g/d for males. Total dietary fiber content was 62 g/day vs control. Both diets were designed in agreement with the Nordic Nutrition Recommendations and supplied 2500–2600 Kcal/day for men and 2000–2100 Kcal/day for women, combining foods from plant and animal origins.	8 weeks

Author	Design	Location	N (subject)		n, GM was analyzed	age (years)	BMI (kg/m^2)	Health Status	Intervention	Duration
			pre	post						
Kahleova et al. 2020	opened label-RCT	Columbia	168 (vegan group =84; control group = 84)	115 (vegan group =65; control group = 50)	115 (vegan group =65; control group = 50)	>18	28-40	healthy overweight adult	low fat vegan diet vs control. Vitamin B12 was supplemented for vegan group (500µg/day)	16 weeks
Vitale et al. 2020	DB-RCT Parallel Group	Italy	Mediterannian diet = 16; Control Diet (western diet) = 13	Mediterranian diet = 16; Control Diet (western diet) = 13	Mediteranian diet = 16; Control Diet (western diet) = 13	Mediteranian diet = 41.6 ± 12.3; control diet = 45.9 ± 13.0	Mediteranian Diet = 28,9,1±2,3 ; Control Diet = 29,3±3,5)	ow/obese	The control diet was instructed to keep their habitual diet unvaried during the intervention and did not consume extra virgin olive oil Mediterranean Diet was designed to have fruit and vegetable 500gr/day, nuts 30gr/day, refined cereal products replaced with wholegrain products 200gr/day, meat and derived meat products, fish 300gr/day, legumes 200gr/day, extra virgin olive oil	8 weeks
Gao Jian et al. 2021	DB-RCT	China	117 (control group = 58; fried meat group = 59)	117 (control group = 58; fried meat group = 59)	117 (control group = 58; fried meat group = 59)	Control group = 21,73; fried meat group = 21,13	Control group = 26,39; fried meat group = 26,06	healthy overweight adult	Fried meat was provided four times per week in the experimental the group with cooking methods, which was frying at 150 C for <3 min; and boiling, steaming, or dressing with sauce at 100 C in the control group.	4 weeks

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Author	Design	Location	N (subject)		n, GM was analyzed	age (years)	BMI (kg/m^2)	Health Status	Intervention	Duration
			pre	post						
Muralidharan et al. 2021	DB-RCT	Spain	400 (intervention group = 200; control group = 200)	400 (intervention group = 200; control group = 200)	262 (intervention group = 183; control group = 179)	intervention group = 64.3 ± 5.1; control group = 65.1 ± 4.9	intervention group = 33.4; control group = 32.9	ow/ob with metabolic syndrome	intervention group = individualized behavioral support, restricted caloric Mediteranian Diet, and physical activity promotion; control group = information on maintaining ad libitum unrestricted caloric Mediteranian Diet with no advice on weight loss strategies	1 year
Tagliamonte et al. 2021	DB-RCT	Italy	82 (Mediterranian Diet = 43, Control Diet =39)	82 (Mediteranian Diet = 43, Control Diet =39)	82 (Mediteranian Diet = 43, Control Diet =39)	Mediterranian Diet = 43±1,4 ; Control Diet = 43±1,9)	Mediteranian Diet = 31,1±0,5 ; Control Diet = 31,2±2,0)	ow/obese	group 1 = Isocaloric Tailored Mediteranian Diet; group 2 = control	8 weeks
Food groups										
Han et al. 2015	RCT	Korea	fresh kimchi group = 12; fermented kimchi group = 11	fresh kimchi group = 12; fermented kimchi group = 11	fresh kimchi group = 10; fermented kimchi group = 10	30 - 60	fresh kimchi group = 28 ± 2.31; fermented kimchi group = 27.8 ± 2.20	ow/obese	consuming 180 g of fresh or fermented kimchi per day (60 g/pkg × 3 meals)	8 weeks

Author	Design	Location	N (subject)		n, GM was analyzed	age (years)	BMI (kg/m^2)	Health Status	Intervention	Duration
			pre	post						
Moreno et al. 2016	RCT-cross over	Spain	MetS group = 10, control group = 10	MetS group = 10, control group = 10	MetS group = 10, control group = 10	48±2	MetS vs control group: 35,24±4,21 vs 27,52±2,10 (washout period); 34,49±4,17 vs 27,34±2,31 (red wine period); 34,53±4,23 vs 27,27±2,19 (de-alcoholized red wine period)	metabolic syndrome	Divided into three periods; the first period was the washout period (participants did not consume any red wine), the second period was drunk only red wine (272 ml/d), the third period was drunk de-alcoholized red wine (272 ml/d)	10 weeks (two weeks/15 days of washout period, followed by two intervention periods of 30 days each)
Lambert et al. 2017	DB-RCT	Canada	53 (pea fiber group = 29; placebo group = 24)	44 (pea fiber group = 22, placebo group = 22)	pea fiber group = 22, placebo group = 22	44±15	33,4±1,3 (PG); 32,8±1,3 (Pea fiber group)	obesity	The pea fiber group received 15g/d pea fiber supplementation with the dose was increased incrementally during the first 3 weeks of the study (week 1 ¼ 5 g/d; week 2 ¼ 10 g/d; week 3 ¼ 15 g/d. Pea fiber is packaged in wafers containing 5 g/serving of yellow pea fiber vs placebo group received an isocaloric dose of control wafers with no pea fiber	12 weeks
Roager et al. 2017	DB-RCT crossover	Denmark	60	60	50 (men = 18; women = 32)	20-65	25-35	ow/ob at risk of developing metabolic syndrome	group 1 = whole-grain ≥ 75 gr/day; group 2 = < 10gr/day of refined grain	8 weeks for each group, with washout period 6 weeks

Author	Design	Location	N (subject)		n, GM was analyzed	age (years)	BMI (kg/m^2)	Health Status	Intervention	Duration
			pre	post						
Food nutrients										
Chambers et al. 2019	DB-RCT-cross over	UK	14	12	12	18-65	29,8±0,9	ow/obese	20 g/day of inulin , 20 g/day inulin-propionate ester / IPE (14,6 g/day of inulin and 5,4 g/day of esterified propionate vs 20 g/day of cellulose (placebo - negative control)	42 days each in random order. The washout period for the next intervention was carried out for 28 days
Kjolbaek et al. 2019	Opened label RCT-cross over	Denmark	30	27	27 (completed all study interventions, AXOS, and PUFA intervention)	18 - 60	25-40	central obese and one criterion of metabolic syndrome	phase 1 (AXOS intervention) consumed a powder supplement of 15 g of wheat bran extract with 4 biscuits/cracker per day; phase 2 (PUFA intervention) consumed fish oil supplement (capsules), containing 3,6 g/d g of N-3 PUFA	12 weeks (two diet periods of 4 weeks each separated by a 4-week washout period)
Guevara et al. 2020	DB-RCT	Mexico	45 (PG = 23; GTG = 22)	45 (PG = 23; GTG = 22)	45 (PG = 23; GTG = 22)	20-60	PG = 34.5 ± 0.98; GTG = 34.6 ± 0.86	obese with insulin resistance	The subjects were randomly selected to form part of the placebo group (PG) or the genistein-treated group (GTG) with genistein capsules (50mg/day)	8 weeks
Probiotic										

Author	Design	Location	N (subject)		n, GM was analyzed	age (years)	BMI (kg/m^2)	Health Status	Intervention	Duration
			pre	post						
Rajkumar et al. 2014	DB-RCT	India	placebo (n = 15), VSL#3 probiotic capsules (n = 15), omega-3 fatty acid capsules (n = 15), or omega-3 capsule + VSL#3 probiotic capsule (n = 15)	placebo (n = 15), VSL#3 probiotic capsules (n = 15), omega-3 fatty acid capsules (n = 15), or omega-3 capsule + VSL#3 probiotic capsule (n = 15)	placebo (n = 15), VSL#3 probiotic capsules (n = 15), omega-3 fatty acid capsules (n = 15), or omega-3 capsule + VSL#3 probiotic capsule (n = 15)	40-60	± 28,79 (range 27-30)	obese, dyslipidemia and insulin resistance	Group 1 = nothing; group 2 = 1 capsule probiotic of VSL#3 everyday; group 3 = 1 capsule of omega 3 everyday; group 4 = 1 capsule of omega 3 and VSL#3 probiotic everyday	6 weeks
Simon et al. 2015	DB-RCT	Jerman	21	21	21 (men = 10, women = 11)	lean group = 49 ± 7; obese = 51 ± 7	lean group = 19-25, obese group = 30-45	obese	placebo group = receive Nutraceutix capsule placebo; intervention group = Nutraceutix capsule contain 10^10 cells of L. reuteri	8 weeks
Depomnier et al. 2019	DB-RCT	Belgium	40	32 (n placebo = 11; n pasteurized = 12; n alive = 9)	32	18-70	placebo = 37,63±5,82; pasteurized = 39,81±4,77; alive = 36,82±3,68	ow/obese with insulin resistance and metabolic syndrome	Alive *A. muciniphila* (live 10^{10} bacteria per day); pasteurized *A. muciniphila* (pasteurized 10^{10} bacteria per day); Placebo	12 weeks
Teronio et al. 2019	DB RCT-crossover	Spanyol	53 (group 1 = 28, group 2 = 25)	53 (group 1 = 28, group 2 = 25)	53 (group 1 = 28, group 2 = 25)	18-65	> 30	obese with metabolic syndrome	placebo group = maltodextrin; intervention group = probiotic *Lactobacillus spp* 9 log10 cfu/capsule, 1 capsule/day, with wash out periode (6 weeks)	12 weeks

IPE: inulin propionate ester, MD: Mediteranian Diet, CD: Control Diet, MFD: Multi Functional Diet, HS: high saturated fat diet, HC: High Carbo, HGI: High glycemic index, HF: high fiber, AXOS: arabinoxylan oligosaccharides, ow/ob = overweight/obese

Table 2. Primary Outcome for Gut Microbiome Following Dietary Interventions (Alpha-Beta Diversity)

Author	Health Status	Dietary pattern	Food Groups	Food Nutrient	Probiotic	Dietary pattern	Food Groups	Food Nutrient	Probiotic
		Alpha Diversity				Beta Diversity			
Fava et al. 2013	Obese Mets	-				-			
Rajkumar et al. 2014	Ob, dislipid, IR				-				-
Han et al. 2015	Ow/ob		-				-		
Simon et al. 2015	Ob				Not changed				Not changed
Moreno et al. 2016	Mets		-				-		
Lambert et al. 2017	Obesity		-				-		
Marungruang et al. 2017	Obese	Not changed				Not changed			
Roager et al. 2017	Ow/ob Mets risk		Not changed				-		
Chambers et al. 2019	Ow/ob			Changed				-	
Depomnier et al. 2019	Ob, IR. Mets				-				-
Kjolbaek et al. 2019	Healthy ow			Not changed				Changed	
Guevara et al. 2020	Ob IR			Changed				Changed	
Teronio et al. 2019	Mets				Not changed				Not changed
Kahleova et al. 2020	Healthy ow	Not changed				-			
Vitale et al. 2020	Ow/ob	Changed				-			
Gao Jian et al. 2021	Healthy ow	Changed				Changed			
Muralidharan et al. 2021	Mets	Not changed				Not changed			
Tagliamonte et al. 2021	Ow/ob	-				-			

Ow/ob: overweight/obese, Mets: metabolic syndrome, IR: insulin resistance

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Anti-Diabetic Effects of *Hibiscus* spp. Extract in Rat and Mice Models: A Review

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ABSTRACT
Diabetes mellitus, a chronic metabolic disease characterized by sustained hyperglycemia, has become a worldwide concern due to the upward trend of recorded cases each passing year. It is one of the leading causes of death in the world. Medication for the management and treatment of diabetes is neither affordable nor accessible in most parts of the Philippines thus raising the need for cost-effective alternatives. Plant extracts have long been used as a treatment for a variety of diseases. One of the plants to display biological activity is Hibiscus spp. It is used to treat a variety of diseases and has steadily gained recognition for its anti-diabetic properties. Several of its plant parts such as the leaves, flowers, and calyces had been used in laboratory models of type 1 and 2 diabetes mellitus. However, methods of extracting biologically active components of the plant vary and yield different results depending upon the concentration and temperature of the extraction procedure. Furthermore, it has shown hypoglycemic effects comparable to commonly used drugs in the treatment of diabetes such as metformin and glibenclamide. Although these studies suggest the efficacy of Hibiscus spp. extract as an antidiabetic agent, it still warrants further clinical trials to establish its efficacy and limitations.

Keywords: diabetes mellitus, Hibiscus spp., plant extract, herbal medicine, mice, rats

BACKGROUND

Diabetes mellitus (DM) is a chronic metabolic disease characterized by impaired insulin production and variable degrees of peripheral insulin resistance resulting in increased blood glucose. Sustained hyperglycemia, the primary clinical manifestation of diabetes, is indicated in the development of complications in the circulatory system, kidney, eyes, and nerves which could be fatal if left untreated.[1] It is considered one of the most common metabolic diseases diagnosed in companion animals and humans alike. According to the International Diabetes Federation, 537 million adults aged 20-79 years old are living with diabetes, and it is estimated to rise to 643 million by 2030. Additionally, over 3 in 4 adults affected by diabetes are from low- and middle-income countries with the Philippines ranking 5th in the number of diabetics in the Western Pacific in 2019 while data from the Philippines Statistics Authority ranked diabetes as the 5th leading cause of mortality in the country in 2021.[2]

Treatment of diabetes includes weight reduction, diet modification, insulin, and oral hypoglycemics. However, medication and management of diabetes are costly with an estimated cost of $92 billion in healthcare and productivity loss. A cross-sectional study conducted on the availability and affordability of 15 commonly prescribed antidiabetic drugs in the Philippines such as acarbose, linagliptin, sitagliptin, insulin, metformin, dapagliflozin, and empagliflozin showed that antidiabetics had an 18.3% availability nationwide which is below the 80% ideal availability set by the World Health Organization. Additionally, originator brand standard treatments for diabetes cost more than one day's salary for the lowest-paid government workers. Filipinos are considered to have substandard access to antidiabetic medicine due to low availability and affordability thus raising the need for cost-effective alternatives.[3,4]

Herbal medicine such as *Momordica charantia, Gymnema sylvestre*, *Hoodia gordonii*, and *Opuntia spp*. has long been used as a non-prescription treatment for diabetes, but there are only a limited number of herbal medicines that have been well characterized with extensive clinical trials as compared to Western drugs.[5,6] One of the plants to display hypoglycemic properties is *Hibiscus* spp., commonly known as "Gumamela" in the Philippines. It is a popular ornamental plant known for its colorful hues of red, orange, yellow, white, and pink. Its flowers can be consumed fresh or cooked while its leaves can be brewed to produce tea. Moreover, it has been cited in various studies for its medicinal properties and has also been consumed as an herbal tea for a variety of ailments such as dysentery, bronchitis, high blood pressure, and constipation.[7] This review aimed to

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update the knowledge about the therapeutic effects of *Hibiscus* spp. extract in diabetes and its comorbidities based on rats and mice study. It has been careful analysis of the scientific literature in several works for its anti-diabetic properties which provide an opportunity for the development of complementary herbal treatment for the management of Diabetes mellitus.

METHODS

This literature review is from different academic research papers. After collecting the articles, analyze each one by breaking it down and identifying the important information and then synthesize and identify the conclusions that can be drawn.

DISCUSSION

Sources of Glucose

Carbohydrate metabolism

Carbohydrates are energy-rich organic biochemical compounds that can be categorized into four main types according to their structure, namely: monosaccharides, disaccharides, oligosaccharides, and polysaccharides.[8] Large aggregates of carbohydrates are not absorbed in the body thus it is metabolized by a series of biochemical processes to reduce them to monosaccharides that can be utilized by the body. The process of metabolism starts in the oral cavity wherein mechanically degraded food meets saliva containing the enzyme, salivary a-amylase, to form a bolus. a(1 → 4)-glycosidic linkages are hydrolyzed by this enzyme to yield maltose, a disaccharide, but this only accounts for approximately 30% of hydrolyzed polysaccharides.[9] Absorption of monosaccharides results in a postprandial increase in blood glucose levels.[10] Glycogenolysis is the process whereby glycogen, the primary carbohydrate stored in the skeletal muscles and liver, is broken down to glucose at periods wherein blood glucose falls below the normal reference range. It starts when the enzyme glycogen phosphorylase is activated by glucagon.[11] Gluconeogenesis is a pathway wherein glucose is synthesized from non-carbohydrate metabolites. The process begins when pyruvate is converted to oxaloacetate by pyruvate carboxylase followed by the conversion of oxaloacetate to malate-by-malate dehydrogenase.[12,13]

To maintain normal function of the body, circulating glucose or plasma glucose must be kept at a certain level. It is regulated by a network of hormones and neuropeptides released largely by the brain, liver, intestine, muscles, adipose tissues, and most notably the pancreas. The pancreas is considered an endocrine and exocrine gland located in the abdominal cavity. Its endocrine function is for enzyme production to aid with digestion.[14] The amount of circulating glucose is primarily regulated by the opposing actions of insulin and glucagon. Insulin secretion is stimulated in response to hyperglycemia to lower blood glucose.[15] After insulin release, cells of insulin-sensitive peripheral tissues located abundantly in skeletal muscles increase the uptake of glucose. The feedback mechanism between insulin and glucagon constantly adjusts according to the metabolic demands of the body until normoglycemia is achieved.[16]

Diabetes Mellitus

Type 1 Diabetes Mellitus

Type 1 DM is a progressive metabolic disease largely attributed to selective autoimmune destruction of pancreatic β-cell although a small number of cases are not caused by autoimmune destruction and are idiopathic.[17] Pathogenesis of type 1 DM may be influenced by environmental factors including reduction in gut microbiota, diet, obesity, toxins, and viruses that may either destroy β-cells directly or indirectly by triggering an immune response and several genetic factors.[18] The disease progresses sub-clinically over months or years until β-cell impairment significantly affects insulin concentration resulting in inadequate control of plasma glucose.[19] In addition to the destruction of pancreatic β-cells, there is also increased secretion of glucagon by pancreatic a-cells which exacerbates hyperglycemia and metabolic defects. This is followed by the development of diabetic ketoacidosis wherein the body compensates for the loss of intracellular glucose by breaking down fats resulting in the release of gluconeogenic substrates, mobilization of free fatty acids, and excess production of ketones in the body. Furthermore, decreased insulin triggers the production of counterregulatory hormones that suppress glucose metabolism in peripheral tissues. Deficiencies in insulin and excess glucose contribute to impairments in lipid, glucose, and protein metabolism by various organs resulting in a multisystemic disturbance.[20]

Type 2 Diabetes Mellitus

Type 2 diabetes is characterized by defective insulin secretion and insulin resistance, or an impaired response of insulin-sensitive tissues to the hormone.[21] Hepatic insulin resistance results in the inability to regulate hepatic glucose production while peripheral insulin resistance hinders glucose uptake by peripheral

tissues. This leads to the accumulation of glucose in the bloodstream coupled with high levels of insulin; however, as the disease progresses insulin production may decrease due to damage in pancreatic β-cells brought about by overcompensation to insulin resistance.[22] Pathogenesis of type 2 DM is complex and not completely understood. Prolonged hyperglycemia would trigger the same compensatory mechanisms employed in type 1 DM to make up for decreased glucose uptake. Clinical signs often manifest when insulin secretion can no longer sustain insulin resistance. The development of this disease is often linked to obesity, family history, a sedentary lifestyle, and old age.[23]

***Hibiscus* spp.**

Hibiscus is considered the genus with the most diverse vegetative, floral, and canopy expressions in its family, Malvaceae.[24] It is an evergreen shrub that can grow up to 8 ft tall in the wild with a light-gray bark that is easy to peel and smooth.[25] One fruit may contain up to 20 brown kidney-shaped seeds. The capsule splits open when the fruit is mature and dry.[26] Hibiscus is native to tropical Asia, but it can be traced back to ancient Egypt and across China through plant anatomy, iconography, published literature, and archaeological records. It naturally grows in warm temperate tropical and subtropical regions in the world, but it is now commonly planted as a flowering shrub throughout the world, especially in China, India, Pakistan, South Indian Islands, and the Philippines.[27]

Hibiscus spp is now widely cultivated for its flowers, fruits, and calyces that may be used as an ornament, medicine, and food source. Plant parts may be prepared fresh or processed to consume as a food product with almost all parts of the plant considered edible. Its flowers have been widely incorporated in some beverages while its seeds are roasted to be eaten alone or with other meals. Its leaves and shoots can be eaten raw or cooked and prepared as a condiment or ingredient in salads. Traditionally, it has been used to treat colds, loss of appetite, and respiratory disorders. It has also been extensively utilized for its diuretic, laxative, and expectorant properties in traditional medicine. Furthermore, it was noted that *Hibiscus* spp has emmenagogue effects that can stimulate menstruation and cause abortion.[28] Proponents of traditional Chinese medicine believe that it can be used to treat a variety of diseases including diabetes.[29] Some of its medicinal properties can now be backed up by modern studies. Among its species, *H. sabdariffa, H. tiliaceus, H. rosa-sinensis,* and *H. taiwanensis* have shown antidiabetic properties in studies using in-vivo models.

Chemical Compounds and Mechanism of Action

The plant is made up of approximately 15-30% plant acids including citric, malic, tartaric acids, allo-hydroxycitric acid, lactone, and Hibiscus acid which is specific to the plant. It also contains alkaloids, L-ascorbic acid, anthocyanin, Beta Carotene, Beta-sitosterol, citric acid, polysaccharides arabians, arabinogalactans, quercetin, gossypetin, and small quantities of galactose, arabinose, glucose, xylose, mannose, and rhamnose.[30] The main constituents of *Hibiscus* spp in relevance to its pharmacological activity are organic acids, anthocyanins, and flavonoids. Studies show that calyces of *Hibiscus* spp are rich in polyphenol and flavonoids, substances that are known for their antioxidant properties. Among the phenols found in calyx extract of the plants were anthocyanins such as sambubioside, cyanidin-3- sambubioside, and delphinidin-3-glucoside while flavonoids consist of hisbiscetin and gossypetin with their respective biosides.[31,32] Leaves of *Hibiscus* spp have been found to contain β-sitosteryl-β-d-galactoside while flower extracts yielded luteolin and quercetin. Its polyphenol content had been indicated to reduce blood glucose and increased plasma insulin levels in diabetic rats.[33] These compounds are considered natural enzyme inhibitors of intestinal a-glucosidase and pancreatic a-amylase resulting in reduced postprandial glucose production.[34]

The 3, 4, 6, 8-tetrahydroxy flavonol-5 - methyl ether 7-O-neohesperidoside, a flavanol biocide, showed significant hypoglycemic activity comparable to glibenclamide, but its exact mechanism of action is still unknown.[26,35] Furthermore, quercetin, hibiscetin, gossypetin, and protocatechuic acid are potent phosphoenolpyruvate carboxykinase (PEPCK) enzyme inhibitors compared to metformin, a common drug used for the management of type 2 diabetes.[36] PEPCK enzyme is responsible for decarboxylation and phosphorylation of oxaloacetate to phosphoenolpyruvate. It is considered a rate-limiting step in gluconeogenesis since it bypasses the thermodynamically unfavorable conversion of pyruvate to phosphoenolpyruvate. In a study conducted on streptozotocin-diabetic mice models, silencing of PEPCK liver enzyme in hyperglycemic mice resulted in a 40% reduction of fasting blood glucose 2 days after initial treatment which suggests that expression of PEPCK regulates the rate of glucose production through gluconeogenesis.[37]

Extraction Method

Bioactive compounds are extracted from plant material through different extraction techniques depending upon the desired compounds to be isolated. The successful methods in the extraction of bioactive

compounds are Soxhlet, heat reflux, hydro distillation, and maceration.[38,39] Solvent extraction is often the preferred method; however, there may be differences in extract yield, for bioactive compounds are highly dependent upon the nature of the extracting solvent. The solvent, plant part, and extraction method are the basic parameters that influence the extract quality.[40] Methanol and ethanol have been proven as effective solvents for phenolic compounds due to their polarity which can extract both hydrophilic and lipophilic plant parts. Bioactive compounds can be identified and characterized by various plant parts such as leaves, flowers, stems, roots, barks, calyx, and fruits.[38,41] Successful extraction begins with careful selection and preparation of plant samples and a thorough review of the appropriate literature for indications of which protocols are suitable for a particular class of compounds or plant species.

In a study conducted on *Hibiscus calyces*, the influence of three concentrations of 50% aq, 75% aq, and 100% methanol and ethanol solvent on extraction yield was noted. It was observed that a higher concentration of both methanol and ethanol had significantly less extraction yield; however, the total phenolic and flavonoid content of the isolate increased proportionally to the solvent concentration.[42] Furthermore, A study suggests that the temperature at which biochemical compounds are extracted yields varying results. *Hibiscus* calyces extracted with water at 23°C, 50°C, 75°C, and 90°C revealed that extract yield was directly proportional to temperature, but total phenolic and flavonoid content decreased as the temperature increased.[43] The various techniques for extraction of bioactive compounds are shown in Table 1.

Table 1. Extraction methods of *Hibiscus* spp

Plant Species	Plant Part	Extraction methods	References
H. taiwanensis	leaves, fruit, and stem	60% aqueous acetone solution at room temperature for 3 days with occasional shaking and stirring	[44]
H. rosa-sinensis	aerial parts	80% aqueous ethanol yielded 10% crude extract	[45]
H. platanifolius	Bark	90% ethanol on a reflux water bath for 3 hours then concentrated using a rotary flash evaporator until a semi-solid consistency	[46]
H. taiwanensis	Stem	60% aqueous acetone with a 2 mL/g ratio of solvent volume to dry weight	[47]
H. rosa-sinensis	Leaves	80% methanol at room temperature for 7 days with shaking and stirring which yielded a 9.42% w/w of the crude extract	[48]
H. rosa-sinensis	dried ground leaves	methanol in a soxhlet apparatus then the aqueous layer was made alkaline using 5% NaOH to obtain the basic fractions while neutral fractions were obtained by neutralizing the aqueous layer with H_2SO_4	[49]
H. surattensis	Leaves	extracted for 24 hours using 96% ethanol by maceration method and further concentrated using a rotary evaporator	[50]
H. sabdariffa	Leaves	Use cold water with powdered leaves for 24 hours then evaporated using water bath evaporation. And use boiled water for 30 minutes. The sample was then left to soak for 24 hours	[51]
H. cannabinus	pulverized leaves	submerging the sample in 1.5 mL methanol for 8 days	[52]
H. rosa-sinensis	Flower	defatted using petroleum ether in a soxhlet apparatus at 60-80°C then extracted using chloroform, ethyl acetate, and then 95% ethanol.	[53]
H. tiliaceus	Flower	defatting using petroleum ether at 60-80°C which is then followed by further extraction using methanol	[54]
H. rosa-sinensis	Flower	using ethanol at 60-80°C for 48 hours	[55]
H. sabdariffa Linn	Petal	boiled with water at a concentration of 2g/200 mL and was further concentrated using an evaporator until its volume reached 10 mL	[56]
H. sabdariffa Linn	Flower	ethanol which had a yield of 45%. It was further concentrated using a rotary evaporator at 40° C	[57]

Plant Species	Plant Part	Extraction methods	References
H. sabdariffa	Calyx	utilized 1 L of distilled water to extract powdered calyxes for 48 hours	[58]
H. sabdariffa	Calyx	boiled in 50 mL methanol at 60°C for 30 minutes. The extracts were filtered, and the same procedure was repeated twice. It was further partitioned with ethyl acetate	[60]
H. sabdariffa Linn	Calyx	ethanol by stratified percolation.	[61]

Experiments on anti-diabetic properties of Hibiscus spp.

Experiments on Type 1 Diabetes Mellitus

The commonly used model for type 1 DM is the non-obese diabetic (NOD) mice. NOD mice develop insulitis at 3-4 weeks of age. Its pancreatic islets are infiltrated by CD4 and CD8 lymphocytes through a process that has immunological and pathophysiological similarities to human type 1 DM.[18] The results of several studies are summarized in Table 2. Type 1 DM caused the person's body reduces very little or no insulin.[62] Type 1 DM, there is autoimmune destruction of the β-cells of the Langerhans islets in the pancreas. Consequently, it reduces or even inhibits insulin secretion by these cells. The summary results showed that the administration of Hibiscus extracts significantly lowered the level of plasma glucose, decreased triglycerides, and improved insulin sensitivity. This effect may be through the stimulation of β-cells of islets of Langerhans secretion of insulin or enhanced transport of blood glucose to the peripheral tissues.[64]

Table 2 Summary of studies conducted on hypoglycemic properties of *Hibiscus* in mice and rat models of type 1 DM

Animal model	Plant Species	Plant Part	Type Extract	Parameters examined				Reference
				Plasma glucose	Plasma insulin	Body weight	triglycerides	
Non-obese diabetic mice	*H. rosa-sinensis*	leaf	Basic ethanol	Decreased	Increased	Increased	Decreased	[49]
			Neutral ethanol	Decreased	Increased	Increased	Decreased	
Streptozotocin-induced diabetic mice	*H. sabdariffa*	calyx	Ethyl acetate	Decreased		Decreased		[60]
Streptozotocin-induced diabetic rats	*H. taiwanensis*	Stem Fruit leaf	Aqueous acetone	Decreased				[44]
Streptozotocin-induced diabetic rats	*H. taiwanensis*	Stem	Aqueous acetone	Decreased				[47]
Streptozotocin-induced diabetic mice	*H. sabdariffa* Linn	calyx	Ethanol	Decreased				[61]
Streptozotocin-induced diabetic rats	*H. cannabinus*	Leaf	Methanol	Decreased		Decreased	Decreased	[52]
Alloxan-induced diabetic rats	*H. sabdariffa* Linn	Flower	Alcohol	Decreased	Increased	Decreased		[53]
			Ethanol	Decreased		Decreased	Decreased	[57]
Alloxan-induced diabetic rats	*H. rosasinensis*	Flower	Ethanol	Decreased				[55]
Alloxan-induced diabetic rats	*H. esculentus*	Fruit	methanol	Decreased			No significant change	[65]
Alloxan-induced diabetic rats	*H. platanifolius*	Stem	ethanol	Decreased		Increased at 500 mg/kg dosage	No significant change	[46]
	H. sabdariffa	Calyx	Aqueous	Decreased				[49]

Alloxan-induced diabetic rats	Leaf	Aqueous	Decreased	Increased	Decreased	[51]

Experiments on Type 2 Diabetes Mellitus

Type 2 DM decreased insulin sensitivity of tissue especially skeletal muscles or liver and caused high-risk factors of hypertension, obesity, dyslipidemia, and insulin resistance.[62] A study conducted by [45] on ethanolic extracts of aerial parts of *H. rosa-sinensis* (HRSAE) administered orally to streptozotocin-induced diabetic mice showed decreased blood glucose to near control level in groups treated with 500 mg/kg HRSAE. Furthermore, urea, uric acid, creatinine, plasma protein, and alanine transaminase (ALT) were lower in treated mice compared to diabetic groups. These are common markers used to determine liver and kidney function in cases of diabetes mellitus, for liver and kidney function is often compromised due to the body's compensatory mechanism to sustained hyperglycemia.[63] This is further supported by a study conducted by[48] that histopathological examination of diabetic rats treated with the extract appeared mostly normal with minimal signs of degeneration while kidney samples had decreased pathological alterations compared to that of the diabetic control group. Similar results were seen in a study of ethanolic leaf extract of *H. surattensis* with those given 300 mg/kg extract having blood glucose comparable to mice treated with 13 mg/kg acarbose, an antidiabetic agent used in the management of hyperglycemia by delaying glucose absorption.[66] A study conducted by[67] found that treatment of pregnant streptozotocin-induced diabetic Wistar albino rats with oral *H. rosa sinensis* flower aqueous extract did not modify blood glucose in treated groups. This is attributed to the severity of the disease suggesting that treatment with Hibiscus extract may only be beneficial in moderate cases of hyperglycemia. The results of several studies confirming the benefits of *Hibiscus* extracts are summarized in Table 3. The administration of *Hibiscus* extracts significantly lowered the level of plasma glucose. Some studies indicate that the administration of *Hibiscus* effectively reduces the level of malondialdehyde as a marker of oxidative stress.[68] Oxidative stress has a two-way mode of action in diabetes that reduces the response of the body's tissues and weakens insulin secretion to its actions, consequently leading to the formation of Type 2 DM. It has been noticed that the metabolic pathways that contribute to the increased formation of oxidative stress in diabetics are the pathways of sugar and fat metabolism.[62] On Type 2 DM mice and rat modelss, it can be concluded that the β-cell dysfunction may be reversible.

Table 3. Summary of studies conducted on hypoglycemic properties of *Hibiscus* in mice and rat models of type 2 DM

Animal model	**Plant Species**	**Plant Part**	**Type Extract**	**Parameters examined**				**Reference**
				Plasma glucose	**Plasma insulin**	**Body weight**	**triglycerides**	
Streptozotocin-induced diabetic mice	*H. rosa-sinensis*	Aerial parts	Ethanol	Decreased				[45]
			Methanol	Decreased				[48]
Streptozotocin-induced diabetic rats	*H. sabdariffa* Linn	Flower	Aqueous	Decreased		No significant change		[56]
Streptozotocin-induced diabetic mice	*H. tiliaceous*	Flower	Methanol	Decreased		Increased		[54]
Glucose-loaded Swiss Webster mice	*H. surratensis*	Leaves	Ethanol	Decreased				[50]

Recommended Dosage

Available toxicological data on *Hibiscus* spp. is limited, but infusions and aqueous extracts are generally considered safe as it has a long-standing history in food and medicine. Studies conducted on extracts of *H. sabdariffa* Linn. showed that it is non-toxic with a high margin of safety. In animal models, it could be given at 150-180 mg/kg/BW per orem without signs of adverse side effects in 3 weeks with LD50 between 2000-5000 mg/kg/day.[69] Another study in animal models stated that consumption of *Hibiscus* tea had no side effects on the liver and kidneys given that it does not exceed 5000 mg/kg/day.[70] However, an increase in liver enzymes and kidney parameters was observed in laboratory mice given dried *H. sabdariffa* Linn. calyxes alcoholic and

water extract at doses of 300 mg/kg/bw over a BW3-month period. This suggests that at high dosages, *Hibiscus* is hepatotoxic. Additionally, an increase in uric acid was also noted in rodents at high dosages which may be attributed to anthocyanins that are responsible for the pigment in flowers of *Hibiscus*.[57,59]

CONCLUSIONS

Studies on *Hibiscus* spp. plant extracts show that the isolation of biologically active compounds is affected by the solvent type, concentration, and temperature during extraction. The extract yield is directly proportional to the concentration and temperature of the solvent; however, the biologically active compounds such as phenols and flavonoids decrease as concentration and temperature increase. Furthermore, the plant's extracts have shown considerable anti-diabetic properties by reducing blood glucose and other toxic metabolic waste, such as uric acid, urea, creatinine, and alanine transaminase which are products of the body's compensatory mechanisms to hyperglycemia. The hypoglycemic property of *Hibiscus* spp. may be attributed to naturally occurring compounds, glycosides, that inhibit enzymes that are vital for carbohydrate metabolism and gluconeogenesis. Pancreatic a-amylase and intestinal a-glucosidase are inhibited during carbohydrate metabolism resulting in decreased absorption of glucose from the intestines. Additionally, glucose production from gluconeogenesis is reduced due to the inhibition of the PEPCK enzyme. Overall, it has shown great potential as a complementary treatment for management of diabetes; however, clinical studies lack rigorous research as to its biochemical compounds that may contribute to management of the disease. Additionally, animals used in studies are often limited to chemical induction models of diabetes with most studies using alloxan or streptozotocin induced diabetic mice. There is also limited research as to its toxicological properties and margin of safety. Further research is warranted to establish efficacy and limitations of *Hibiscus* spp. plant extract as an antidiabetic agent.

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Determining the Valid Tools to Screen Malnutrition in Cancer Patients: A Comparison to Patient Generated-Subjective Global Assessment (PG-SGA)

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ABSTRACT

Background: Nutrition screening tools are necessary to predict the risk of malnutrition for cancer patients.
Objectives: This study aimed to investigate the validity of nutrition screening tools in identifying malnutrition among cancer patients.
Materials and Methods: This cross-sectional study involved 175 oncology patients in Dr. Sardjito General Hospital. Malnutrition risk of participants was screened using Nutrition Risk Screening (NRS) 2002, Simple Nutrition Screening Tool (SNST), Malnutrition Screening Tool (MST), Nutriscore, and the Royal Marsden Nutrition Screening Tool (RMNST). Patient Generated-Subjective Global Assessment (PG-SGA) was used as a gold standard. Nutritional assessments, including Body Mass Index (BMI), Mid-Upper Arm Circumference (MUAC), albumin, hemoglobin, Total Leucocytes Count (TLC), and Hand Grip Strength (HGS), were used to evaluate nutritional status.
Results: The NRS 2002, SNST, MST, Nutriscore and RMNST identified nutritional risk in 64.6%; 58.9%; 49.1%; 30.3%; 84.6%, respectively. The SNST obtained the highest level of AUC discrimination (0.8) compared to NRS 2002 (0.7); MST (0.7); Nutriscore (0.7); and RMNST (0.7). There was a significant association between nutrition screening with nutritional parameters except for TLC (P>0.005). Patients who were at risk of malnutrition had a lower average of objective assessment tools.
Conclusion: All the nutritional screenings were valid to screen for malnutrition risk among cancer patients. Nutritional screening has a strong correlation with nutritional assessment. The lower risk detected by nutrition screening, the poorer the nutrition status measured by nutrition assessments.

Keywords: validity, nutrition screening tools, PG-SGA, cancer

BACKGROUND

Malnutrition is one of the problems faced by hospitalized patients.[1] The incidence of malnutrition among cancer patients was elevated.[2-5] Malnutrition in oncology patients is caused by disease-associated inflammation, effects of therapy, or other mechanisms. This condition, in the long term, leads to decreased body composition and diminished biological function.[5,6] Both of them contribute to anorexia, decreased food intake, as well as elevated metabolism, and increased protein catabolism.

Nutrition screening is an essential step before implementing the Nutrition Care Process on inpatients within 24 hours of admission to identify the risk of malnutrition. Academy of Nutrition and Dietetics (AND) stated that nutrition screening tools must be easy to complete, cost-effective, quick, and able to identify individuals at risk of malnutrition.[7,8] The ESPEN consensus recommends the Nutritional Risk Screening (NRS) 2002 as a good nutrition screening method as has been analyzed by several RCT studies.[9] Other literature reviews found that the Malnutrition Screening Tool (MST) was the nutrition screening tool with the highest ranking on the specific criteria. The Royal Marsden Nutrition Screening Tool (RMNST) was developed through professional consensus by the Department of Nutrition and Dietetics for inpatient use.[10] Nutriscore is a new screening tool recommended by Spanish Oncology Societies, which is the development from MST screening.[11]

In current clinical settings, nutrition screening tools required calculations and data that can only be revealed by skilled healthcare professionals. For these reasons, a new nutritional screening tool has been developed named the Simple Nutrition Screening Tool (SNST), which has been proven valid in detecting

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patients at risk of malnutrition compared with the gold standard, the SGA (sensitivity 91%; specificity 80%).[12] This study aimed to investigate the validity of nutrition screening tools for oncology patients.

MATERIALS AND METHODS

This study was a cross-sectional study conducted in Dr. Sardjito General Hospital, the central hospital in Yogyakarta Province, Indonesia. The study received ethical clearance from the Ethics Committee of the Faculty of Medicine, Universitas Gadjah Mada, Indonesia (KE/FK/0850/EC/201). Written informed consent was obtained from 175 participants, adult patients aged 18-60 years who were admitted to the oncology unit without pregnancy or postpartum conditions. Within 24 hours of hospital admission, the nutrition screening tools were carried out. The nutrition screening tools are Nutrition Risk Screening (NRS) 2002, Simple Nutrition Screening Tool (SNST), the Royal Marsden Nutrition Screening Tool (RMNST), Nutriscore and Malnutrition Screening Tool (MST). We assessed malnutrition status using Patient Generated-Subjective Global Assessment (PG-SGA) as a gold standard.

The new nutritional screening tools for oncology patients, Nutriscore and RMNST, were developed to predict the risk of malnutrition for oncology patients. Nutriscore is a new nutritional screening tool developed from MST screening. There are modifications to the nutrition screening form by adding two additional questions about the tumor site and the oncological treatment.[10] The Royal Marsden Nutrition Screening Tool (RMNST) incorporates parameter that is considered in nutrition screening, such as weight loss during the previous three months and food intake less than 50% in the previous five days. The symptoms that affect food intake, such as mucositis, dysphagia, and nausea, are also included as these have been shown to influence the risk of malnutrition[10]

The SNST, the novel nutrition screening tool developed in Indonesia, is a simple nutritional screening tool with six questions that do not include anthropometric and weight loss measurements. The SNST questions were 1) Does the patient look thin?, 2) Do your clothes feel loose?, 3) Have you recently lost weight unintentionally (6 months)?, 4) Have you decreased food intake during the past weeks? 5) Do you feel weak, sluggish, and not powerful?, and 6) Do you suffer from a disease that results in a change in the amount or type of food you eat?. [12]

Patients were screened upon admission and were identified using each nutrition screening tool's cut-off points, NRS-2002 ≥3; MST ≥2; SNST≥3, NUTRISCORE ≥5, and RMNST ≥4 and were categorized into two groups: not at risk and risk. The RMNST was designed to categorize the patients as well-nourished (cumulative score ≤4), moderately malnourished (score 5-9), and severely malnourished (score >10). The PG-SGA was adapted for the oncology population from the SGA tool. Due to its high sensitivity and specificity, it has been widely used in other oncology and patient settings and has performed well against other tools and is therefore used to cross-validate other screening tools. All the relevant sections of the PG-SGA were completed and summarized to classify the patients as well-nourished (PG-SGA–A), moderately or suspected of being malnourished (PG-SGA–B), or severely malnourished (PG-SGA–C).[10-12]

In order to compare the screening tools, outcome data for PG-SGA and RMNST was categorized into those at risk and not at risk of malnutrition. For PG-SGA data, number of patients falling into classification "B" and "C" of the PG-SGA tool were summed as those patients at risk of malnutrition. While for RMNST data, scores of more than five were classified as those at risk of malnutrition.[10]

Body weight was measured with electronic digital scales, and height was measured by microtoise to the nearest 0.5 kg and 0.5 cm, respectively. The Mid Upper Arm Circumference was measured by measuring the circumference of the upper arm at the middle point between the end of olecranon and the tip of acromion is measured using a standardized tape.[13] Albumin, hemoglobin, and TLC were also performed using secondary data from latest laboratory readings results. The value for TLC, which less than 1,500 cell/mm^3 was classified as malnutrition for both genders. [14] Normal hemoglobin level for males 13 g/dL and females 12 g/dL. [15] Serum albumin levels less than 3,5 g/dL are known as a parameter for malnutrition. [14] Handgrip strength was measured using hand grip strength dynamometer with position of the patient seated with their shoulders adducted, elbows flexed into 90° this measurement was repeated three times then mean was calculated.[16]

Characteristics of patients were presented by descriptive analysis. The sensitivity, specificity, maximum sum of sensitivity and specificity (MSS), positive predictive value (PPV), and negative predictive value (NPV) were determined to compare the accuracy of each screening tool in detecting malnutrition. Discrimination values of AUC determine the accuracy of a nutrition screening tool in detecting malnutrition. Values for each nutrition screening tool were interpreted as acceptable (0.70–0.80), excellent (0.80–0.90), or outstanding or the highest level (>0.90).[17] An independent sample t-test was performed to compare the nutrition screening tools and nutritional assessment. Significance was set by the P-value <0.05 with 95% CI.

RESULTS

In this study, we included 175 patients (42 males and 133 females), predominantly <60 years of age (80.6%). Most of the patients had gynecologic cancer (53,7%) (Table 1). The nutrition screening tools identified patients with risk of malnutrition differently. Figure 1, showed that nutritional screening by the NRS 2002, SNST, and RMNST identified patients who were at risk of malnutrition as 64.5%; 58.9%; 84.5%, respectively, while the MST and RMNST identified patients were only 49.1%; 30.3% respectively.

Table 1 Characteristics of Participants (n=175)

Characteristics	n	%
Sex		
Males	42	24.0
Females	133	76.0
Age (years)		
18-40 years old	22	12.6
41-60 years old	127	72.6
61-80 years old	26	14.9
Education degree		
Elementary	71	40.6
High school	86	49.1
University	18	10.3
Cancer Diagnose		
Head-neck	17	9.7
Breast	5	2.9
Liver	7	4.0
Gynecologic	94	53.7
Lung	15	8.6
Colon and Rectum	15	8.6
Leukemia	19	10.8
Others	3	1.7

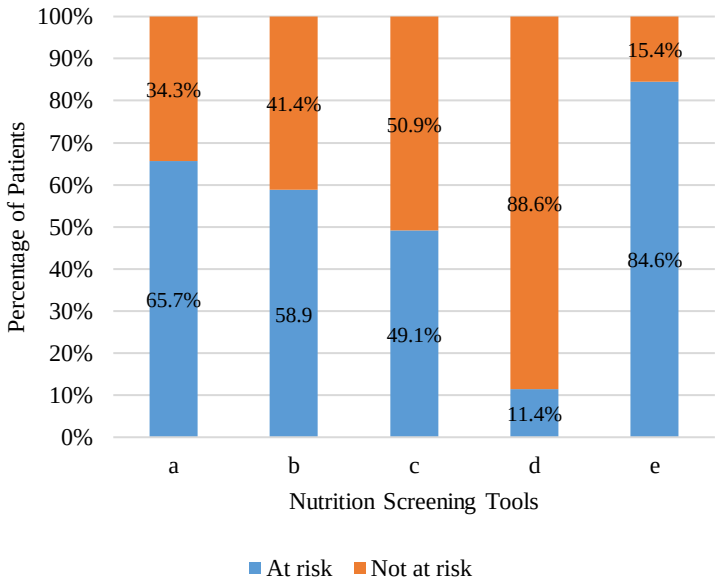

Figure 1. Prevalence Risk of Malnutrition based on Different Nutritional Screening Tool – a: Nutrition Risk Screening 2002; b: Simple Nutrition Screening Tool; c: Malnutrition Screening Tool; d: Nutriscore; e: Royal Marsden Nutrition Screening Tool.

The accuracy of each nutrition screening tool in identifying the risk of malnutrition against PG-SGA is shown in Table 2. The RMNST and SNST had the highest sensitivity, which means these nutrition screening tools are good for detecting malnutrition. Whereas the Nutriscore has high specificity but low sensitivity, The highest MSS (maximum sum of sensitivity and specificity) was achieved by SNST, which means that the higher the MSS value, the better the tool. Table 2 showed that the SNST was to be an excellent nutrition screening tool because it had the highest Area Under ROC Curve (AUC) discrimination.

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Table 2 Accuracy of Screening Tools to Identify malnutrition (as determined by Patient-Generated Subjective Global Assessment)

Nutrition Screening	Sensitivity	Specificity	MSSS	PPV	NPV	AUC (95% CI)
SNST	81.7	90.9	172.6	95.1	69.4	0.8 (0.8-0.9)
MST	64.2	83.6	147.8	89.5	51.7	0.7 (0.6-0.8)
Nutriscore	43.3	98.1	141.4	98.1	44.3	0.7 (0.6-0.7)
RMNST	97.5	43.7	141.2	79.1	88.9	0.7 (0.6-0.7)

SNST: Simple Nutrition Screening Tool, MST: Malnutrition Screening Tool, RMNST: Royal Marsden Nutrition Screening Tool, MSSS: maximum sum of sensitivity and specificity, PPV: positive predictive value, NPV: negative predictive value, AUC: area under the curve.

The association between the nutrition screening tool score with nutritional assessment is shown in Table 3**.** There are significant associations between NRS 2002, SNST, and RMNST with all the nutritional status parameters (p<0,05) except for the TLC. There were no significant associations (p>0,05) between MST and Nutriscore with all nutritional parameters except for the MST with handgrip strength. The analysis also showed that patients at risk of malnutrition had a lower average value for nutritional assessments such as BMI, MUAC, albumin, Hb, and TLC compared with patients who are not at risk of malnutrition.

Table 3 Association Between Nutrition Screening Parameter by NRS 2002, SNST, MST, Nutriscore, and RMNST with Anthropometric and Biochemical Assessment

Nutrition Tool	Screening	Nutritional Parameters					
		BMI (kg/m^2)	MUAC (cm)	HGS	Albumin (g/dl)	Hemoglobin (g/dl)	TLC (cell/mm^3)
NRS 2002	At-risk (n=113)	20.35±4.57*****	23.7±4.24*****	13.56±6.07*****	3.44±0.61*	10.74±1.99	1.810±2.10
	Not at risk (n=62)	23.20±3.27*****	26.46±3.97*****	16.99±7.74*****	3.64±0.74*	11.14±1.86	1.764±2.00
SNST	At-risk (n=103)	20.89±4.65*	24.06±4.39*****	13.65±6.75*****	3.35±0.61*	10.56±2.12	1.719±2.16
	Not at risk (n=72)	22.03±3.86*	25.56±4.12*****	16.38±6.80*****	3.74±0.68*	11.33±1.59	1.900±1.92
MST	At-risk (n=86)	21.68±4.78	24.96±4.25	13.40±6.49*	3.39±0.63	10.55±2.21	1.915±2.39
	Not at risk (n=89)	21.05±3.93	24.39±4.42	16.10±7.03*	3.62±0.69	11.19±1.63	1.677±1.69
Nutri-score	At-risk (n=53)	20.95±3.81	24.46±4.20	13.24±6.60	3.40±0.65	10.70±2.03	1.602±1.37
	Not at risk (n=122)	22.30±5.35	25.15±4.64	15.44±6.93	3.56±0.68	10.96±1.93	1.878±2.33
RM-NST	At-risk (n=53)	21.09±4.53*	24.38±4.45*	14.33±6.75*	3.44±0.67*	10.73±2.02*	1.811±2.22
	Not at risk (n=122)	22.81±3.03*	26.26±3.27*	17.22±7.23*	3.89±0.55*	11.68±1.29*	1.697±0.73

*Significant p<0.05. NRS 2002: Nutrition Risk Screening 2002, SNST: Simple Nutrition Screening Tool, MST: Malnutrition Screening Tool, RMNST: Royal Marsden Nutrition Screening Tool, BMI: body mass index, MUAC: mid-upper arm circumference, HGS: hand grip strength, TLC: total leukocyte count.

Malnutrition is one of the problems for oncology patients. More than 40% of female malignancies are gynecological cancers which is cancer cervix,[16] It appeared the most frequent cancer among females. [17] We found that almost half of the patients were gynecological cancer (53.7%). A previous study found that the peak age group in gynecological cancer was between 45-54 years old as shown in Table 1. Our study showed that the prevalence of malnutrition in oncology patients has ranged from 30%-83%. This discovery is quite high for the prevalence of cancer-related malnutrition.[2-5] As the first step of the nutrition care process, nutrition screening has an important role in detecting the risk of malnutrition before implementing nutritional support.

DISCUSSION

The performance of each screening tool in identifying the risk of malnutrition as determined by the PG-SGA is presented in Table 2. Due to detecting the risk of malnutrition, such a tool would identify all malnourished patients for assessment. The SNST and RMNST have a high sensitivity (81.7%; 97.5%). The MST and Nutriscore were showing high specificity but had low sensitivity (83.6%; 98.1%), indicating that malnourished patients could be overlooked using these nutrition screening tools. Our study found that

according to PG-SGA, the incidence rate of malnutrition in cancer patients was 68.6%. Martins reported that PG-SGA could be demonstrated as a significant association in predicting cancer cachexia and death in oncology patients.[20]

According to van Bokhorst-de van der Schueren et al. a good validity of the screening tools has both sensitivity (Se) and specificity (Sp) of >80%. [21] In this research, a screening tool that was considered into good validity was only the SNST (se 81.7% and sp 90.9%), while MST was fair due to the sensitivity or specificity <80% and both are >50% (se 64.2% and sp 83.6%). Lastly the RMNST and Nutriscore were considered poor due to the sensitivity or specificity <50% (se 97.5% and sp 43.7%; se 43.3% and up 98.1%, respectively). The sensitivity of SNST was higher than the specificity. This follows the theory that a nutrition

screening tool should have high sensitivity to predict more malnutrition risk in patients.[22] The RMNST has the highest sensitivity, but the specificity of the RMNST was poor compared to PG-SGA as a gold standard (43.6%). This would result in the classification of normally nourished patients into the category of malnourished or at risk of malnutrition. Likely, the inclusion of questions within the RMNST that are specifically related to symptoms affecting food intake could contribute to this misclassification. [11]

The MST and Nutriscore demonstrated a specificity of 83.6% and 98.1%, respectively, which was good but lower than previous studies undertaken in the outpatient setting.[11-12] This study showed it to be highly specific because of the high number of false-negative in this both screenings. The false-positive probably contributed to the early detection and diagnosis of cancer which many patients were not at risk of malnutrition but actually were diagnosed with cancer. This finding showed that both screenings are good for catching the actual cause of diseases rather than predicting the presence of malnutrition. Nutriscore focused on the tumor site and treatment for cancer patients, which can present different figures on malnutrition.[11]

The AUC evaluates the tool's ability to discriminate between malnourished and well-nourished participants correctly. It is also useful in determining the performance of the screening tools as compared to PG-SGA. As the new screening tool in Indonesia, SNST has the best performance, which achieved an AUC of 0.8. Based on the reported research by van Bokhorst-de van der Schueren et al, a good validity of the screening tools that have an AUC of >0.8. In our study, we found that a screening tool that was considered good was also only the SNST (0.9), while the others were considered fair due to their AUC ranging from 0.6 – 0.8 (MST 0.7; Nutriscore 0.7; RMNST 0.7). [21] This result is in accordance with the study of Nuraini, who stated that the SNST has a better validity than the NRS 2002 and RMNST.[20] Nutriscore and RMNST also show good performance with AUC >0.7, which would be interpreted as acceptable screening tools to identify malnutrition. A study by Sarasati showed that patients who were at risk of malnutrition based on NRS 2002, SNST, and Nutriscore had lower nutritional assessment compared with patients not at risk of malnutrition.[23]

All of the screening forms included questions about recent weight loss more than 10% in three months would be categorized as severely malnourished. Our study found that mean BMI from at-risk and not at-risk groups was ranged from 20-23 kg/m^2. Bodyweight may also have been influenced in extreme cases by tumor mass and response to treatment. Low initial BMI and more pronounced weight loss in cancer patients strongly correlate with lower survival and worse disease outcomes.[24] These factors make body weight to be a less reliable indicator of malnutrition.

The salient point is the negative energy balance and skeletal muscle loss observed, which is driven by a combination of reduced food intake and metabolic derangements.[26-27] Reduced skeletal muscle mass and function also occur in inpatients with cancer. Our study found that the malnourished group had a HGS range of 13.24-13.65 kgs, while well-malnourished subjects ranged from 15.44-16.99 kgs. According to the European Working Group on Sarcopenia, our subjects were had weak strength, which defined as dynapenia (HGS <30 kg for men and <20 kg for women).[28] Previous study in oesophago-gastric cancer showed that low muscle mass is strongly correlated with malnutrition, such as low anthropometric assessment. [29] Immune function is impaired in malnourished cancer patients and can be used to assess nutritional status. We did not find a significant correlation between nutrition screening tools with TLC. Despite the fact that lymphocyte counts can describe the severity of malnutrition, it depends on some hematological malignancies, immunosuppressive drugs, and infections.[25]

Serum albumin and hemoglobin provide a simple method of estimating visceral protein function and also being part of inflammation suppression. Almost half of our subjects were hypoalbuminemia (48.6%). Hypoalbuminemia in cancer patients supports the possibility of enhanced albumin catabolism in these metabolically affected patients.[30] Our study found that almost all of our cancer patients were anemia with hemoglobin levels ranging 10.55–11.14 g/dl in both malnourished and well-malnourished groups. Both low serum albumin and hemoglobin were also revealed in the previous study.[28-30] Anemia is a common condition in cancer patients associated with most chronic conditions and be a consequence of both myelosuppression of stem cells by tumor cell products and cytotoxic therapy. [24,31]

The search for indicators that reflect nutritional status changes in oncology patients is the most important because by identifying patients with risk of malnutrition before the evident signs such as body weight and weight loss are observed, early nutrition intervention can be established.[32] Our study found a significant association between nutritional screening with nutritional assessment, such as BMI, MUAC, hemoglobin, and albumin serum level, except for the TLC. This result is also in accordance with the previous study.[23] The cancer patient will be inserted with anti-cancer treatments such as chemotherapy, radiotherapy, and surgery. This treatment will make consequences of malnutrition which is characterized by weight loss, anorexia syndrome, and reducing food intake. Our study did not analyze the association between cancer treatment with nutritional status, which may become our limitations. Nevertheless, Nutriscore also included cancer treatment into screening questions to ensure that the severity of malnutrition may occur from the treatment.

A potential strength of our research is that we can present that various nutrition screening tools can be used in clinical settings. Besides, they are significantly associated with all objective assessments. Besides NRS 2002 and MST, which have been recommended for clinical settings, the SNST should be considered to be one of the valid and reliable screening tools to detect the risk of malnutrition for inpatient cancer. Our study revealed that new screening tools for oncology patients, Nutriscore and RMNST, showed good performance for detecting malnutrition and need to be developed more.

CONCLUSION

The prevalence of malnutrition in oncology patients was quite high based on nutritional screening and assessment. All the nutrition screening tools appropriately predict malnutrition in hospitalized cancer patients in Indonesia. Besides nutritional screening, nutritional assessment must be carried out since admission to ensure the severity of malnutrition so early detection can be prevented. Further research should explore the use of nutritional screening and intervention before, during, and after hospitalization to ensure the appropriate nutritional intervention.

ETHICAL STATEMENT

All procedures performed in studies involving human participants were under the ethical standard by the Ethics Committee, Faculty of Medicine, Nursing and Public Health Universitas Gadjah Mada and the approval number was KE/FK/0850/EC/2018. Informed consent was obtained from all participants included in this study.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest. This study was funded by a research grant from Universitas Gadjah Mada.

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Consumption Pattern Score in Cancer Survivor with Chemotherapy Induced Nausea and Vomiting and Non-Cancer at Shelter Houses

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ABSTRAK

Background: One of the most common effects of chemotherapy in cancer survivors is nausea and vomiting. This can affect the diversity of food consumed. Family support and assistance need to be done to increase food intake with one food provision.

Objectives: This study aimed to find out the difference in the consumption pattern score among cancer survivors and non-cancers in shelter houses.

Materials and Methods: This study was a cross-sectional study with a retrospective approach. The criteria of the case subject were undergoing chemotherapy, while the control subject criteria were included in one food supply. The total subject was 66 cancer survivors, with 33 subjects each. This research was conducted from August 2021 until October 2021 at Shelter Houses. The data included the subjects characteristic data, vomit nausea degree data using the Rhodes Index nausea vomiting and retching (RINVR), family support data, food intake data using the food frequency questionnaire (FFQ), and individual dietary diversity score (IDDS) questionnaires. Data collection is done by interviews in person and online. The data collected was analyzed using Chi-Square and bivariate test using Mann Whitney test.

Results: The majority of cancer subjects were aged 40-59 years whereas non-cancer subjects were 20-39 years old. The subjects have special characteristics which are in low financial ability. Consumption of starchy foods ($p<0.001$) and green vegetables ($p<0.006$) in these two group subjects had significant differences. In addition, the consumption pattern score between cancer and non-cancer subjects made significant differences ($p<0.001$).

Conclusion: Average consumption pattern scores showed cancer subjects were lower compared to non-cancer subjects. Thus, consumption patterns in cancer subjects did not variaty compared to non-cancer subjects. It is necessary to conduct further research by analyzing the diversity of food of each subject using a 1x24 hour for 3 days, food access questionnaire and food security.

Keywords: chemotherapy; nausea and vomiting; consumption patterns; family support

BACKGROUND

Cancer is a normal cell that undergoes mutations that cause uncontrolled cell division with other body cells. In addition, cancer can be called a complex disease resulting from various interactions between genes and the environment, and certainly is considered to be one of the leading causes of death worldwide.[1] Based on Basic Health Research, the prevalence of tumors or cancers in Indonesia shows an increase from 1.4 per 1000 population in 2013 to 1.79 per 1000 population in 2018. Factors that influence the increasing incidence of cancer and death are poor diet in individuals.[2]

One of the problems that will arise in cancer survivors is malnutrition since approximately 40-80% of the patients experience decreased appetite and weight loss. This condition can affect treatment outcomes, delay wound healing, worsen muscle function and increase the risk of postoperative complications.[3] However, the most widely used treatment or therapy for cancer survivors is chemotherapy. The treatment mechanism works systemically by killing cancer cells using anti-cancer drugs. The effects of chemotherapy vary greatly from mild to severe, one of the effects is nausea and vomiting or is often called Chemotherapy Induced Nausea and Vomiting (CINV).[3–5] CINV in cancer survivors has 2 phases namely the acute phase and delayed phase. The acute phase occurs within 1-2 hours after chemotherapy and can last up to 24 hours. While the delayed phase occurs for more than 24 hours after chemotherapy, this phase usually begins on the 2nd day after chemotherapy and can last until the 5th day.[6,7]

The effects of nausea and vomiting experienced by cancer survivors will result in loss of appetite, thus will limit their consumption and leading to malnutrition as well as slowing down the recovery process. Cancer

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survivors who experience nausea and vomiting also find it difficult to eat adequately and eventually, only spend on small foods such as bread or biscuits.[7] In addition, the most widely consumed food groups in cancer survivors are cereals and nuts because they are easier to digest by cancer survivors compared to meat, milk, and eggs. Meat and eggs are consumed the least because of the high price that is difficult to access by low-income cancer survivors.[8,9]

Research on consumption patterns with weak economic levels showed that these patients had low diversity of food, especially in animal and vegetable sources. Consumption patterns based on individual dietary diversity scores (IDDS) also indicate a lack of food variety, low food access, and nutritional inadequacy. Several factors that affect consumption patterns include income, education, environment, employment, knowledge, and access to food.[10] Diversity of food is an important indicator because it can assess nutritional status, access to food availability, and socioeconomic level. Dietary diversity among cancer and non-cancer survivors can be measured using IDDS since it can see the food groups that both groups is eating.[8,11]

In 2015 research was conducted in East Java showed that cancer survivors will be more motivated to consume meals if accompanied by a companion. It can affect cancer survivors to increase their appetite, particularly in terms of food diversity. Support provided by the family are given in the form of eating together and accompanying cancer survivors in treatment and medication.[12] Nevertheless, in this study not necessarily all cancer survivors accompanied by a companion have the urge to eat a variety of foods. This study aims to find out the difference in consumption patterns of cancer survivors and non-cancer survivors in low-economic cancer survivors and their relatives, considering both groups have similarities in accessing food, coming from one kitchen and being patients from shelter houses. Families and cancer survivors get help from non-profit organizations that assist in carrying out therapy or treatment. In addition, cancer survivors in shelter houses have special characteristics which are in low financial ability. Food at shelter houses can be served with ready meals and some fresh food.[13]

MATERIALS AND METHODS

This research was conducted on cancer survivors and non-cancer at Rumah Singgah Sedekah Rombongan (RSSR Semarang, Yogyakarta, Malang), Rumah Singgah Sahabat Lestari, Rumah Singgah Peduli, Rumah Singgah Hanum, Yayasan Kanker Indonesia (YKI Semarang dan Yogyakarta), Rumah Sehat Mandiri Yogyakarta, Rumah Imajinasinya Indonesia Yogyakarta, dan IZI Jawa Tengah. This research was held from August 2021 until October 2021 and included in the field of community nutrition with a cross-sectional study under a retrospective approach. All research protocols were approved by the Health Research Ethics Committee (KEPK) Faculty of Medicine, University of Sultan Agung Semarang Number.203/VII/2021/Commission on Bioethics. The minimum sample using unpaired comparative analytics for each subject was 30 people and the total sampling obtained was 33 people who filled out the informed consent.[14]

The inclusion criteria of cancer and non-cancer groups were women aged 20-59 years, including in a low-income family according to the Central Bureau of Statistics in Indonesia year 2016, who can communicate clearly and cooperatively, and were willing to become research respondents.[15] In addition, subjects from non-cancer group were as the same food supply/kitchen as cancer survivors. Whereas the cancer survivor group had undergone chemotherapy and being a patient assisted by shelter houses. Exclusion criteria in this study were the subject moved away from the shelter houses, passed away prior to the data being completed and withdrew their informed consent to participate in the study.

The independent variable in this study was consumption patterns, that is eating habits which include diversity, types or kinds of food. Determination of consumption patterns can show the nutritional value of food, nutritional adequacy, food availability, variety and the combination of food types. Firstly, the type of food and the frequency of consumption in the past month was obtained using the Food Frequency Questionnaire form. The next step was this data then converted into Individual Dietary Diversity Score (IDDS) questionnaire. IDDS consists of nine food groups, which are foods made from flour, cereals and legumes, nuts/seeds, all dairy products, animal (meat, fish), eggs, green vegetables, vegetables and fruit sources of vitamin A, vegetables and other fruit. If the subjects consumed each nine food groups, then it scored 1 in each group, and if not consumed then it scored 0. IDDS was categorized as a variety if the total score was five or above, and was not variety if the score was under five. [11,16–18] The dependent variable in this study was cancer and non-cancer survivors. The data collected including the subject's characteristics, nausea and vomiting information, family support, and food intake for each subject. Data collection through in-person interviews and using online. Interviews via online were conducted by contacting the subject remotely using telephone or WhatsApp. Data collection were carried out by researchers and enumerators, with a nutritional background knowing how to FFQ intake and interviews regarding the degree of nausea and vomiting. Due to time

constraints, communication tools such as handphone, and the facilities needed for data collection were carried out directly to the shelter houses. In order to Covid-19 restriction, health protocols were carried out during direct interviews such as using complete personal protective equipment, administering vaccines and antigen swabs, and before contacting the subject, cleaning/washing hands. Time allocated for researchers and enumerators needed to interview was approximately 45 minutes for each respondent.

Characteristics data on both cancer and non-cancer group consisted of name, address, education level, income, occupation, and access to food availability. Access to food availability aims to find out in terms of the distance from the house to the place of buying and selling and the ease of getting food availability. The category for easy access to groceries was the distance from home to the market that is close and affordable. In addition, data on the cancer group included the type of therapy, medical diagnosis, year of diagnosis, and feelings when interviewed.

Nausea and vomiting data were viewed using the Rhodes Index of Nausea Vomiting and Retching (RINVR) which aimed to determine the degree of nausea and vomiting caused by chemotherapy, consisting of 8 questions that have been validated in previous studies. The RINVR questionnaire score categories were normal (0), mild (1-8), moderate (9-16), severe (17-24), and very severe (25-32). The family support data aimed to find out the role of relatives to motivate cancer survivors in their daily lives. This questionnaire consists of 15 questions regarding emotional support, instrumental support, information/knowledge support, and appreciation/appraisal support. In addition, this questionnaire has been validated in previous studies. The categories of family support scores determined were less (15-30), sufficient (31-45), and good (46-60).[19–21]

The data processing and analysis were carried out using a Statistical Program for Social Science (SPSS) as computer program. The research data were statistically tested with univariate and bivariate analysis. Univariate analysis was used to determine the description of the characteristics of the subject and each variable, whereas bivariate analysis was used to determine differences in consumption patterns of cancer and non-cancer survivors using the Mann Whitney Test.

RESULTS

Characteristic Cancer Survivors and Non-Cancer

Characteristics of cancer survivors and non-cancer include age, education level, gender, access to food availability, and income. Table 1 presented the distribution of characteristics of cancer and non-cancer survivors**.**

Table 1. Characteristic Cancer Survivors and Non-Cancer

Characteristic	Cancer		Non-Cancer		p-value
	n	%	n	%	
Age					
20 – 39 year old	5	18.5	22	81.5	<0.005*
40 – 59 year old	28	71.8	11	28.2	
Education Level					
University	4	36.4	7	63.6	0.030
Senior High School	4	33.3	8	66.7	
Vocational School	1	20	4	80	
Junior High School	9	60	6	40	
Elementary School	13	65	7	35	
No School	2	66.7	1	33.3	
Access to Food Availability					-
Easy to access	21	64	21	64	
Hard to access	12	36	12	36	
Income					
Rp.900,000– Rp.1,500,000	33	100	33	100	-
Nausea and vomiting					-
Mild	6	18.2%	-	-	
Moderate	10	30.3%	-	-	
Severe	17	51.5%	-	-	

The factor that most influences consumption patterns is socioeconomic, of both subjects include low economics and education level or knowlegde.Age and education level in cancer and non-cancer subjects were different significantly (p-value <0.05). The majority of cancer subjects were 40-59 years old (71.8%) while non-cancer subjects were 20-39 years old (81.5%). Most cancer subjects were elementary school graduates (65%) while non-cancer subjects were high school graduates (66.7%). This data shows that foodstuffs are

easily accessible (64%). In terms of the degree of nausea and vomiting experienced by subjects, more than half of the subjects were severe (51.5%) because of the effects of chemotherapy.

Family Support Data on Cancer Survivors

Family support data for non-cancer subjects consisted of emotional support, instrumental support, information/knowledge support, and appreciation/assessment support. Table 2 presents the distribution of data on family support provided to cancer subjects.

Table 2. Family Support Data

Characteristic	Frequency	n	%
Emotional Support			
Family assistance in care	Never	3	9.1
	Occasional	6	18.2
	Frequently	6	18.2
	Always	18	54.5
Family attention during treatment	Occasional	1	3
	Frequently	2	61
	Always	30	90.9
Trying to listen to complains	Occasional	3	9.1
	Frequently	4	12.2
	Always	26	78.8
Family assistance to meet patient needs	Occasional	1	3
	Frequently	4	12.1
	Always	28	84.8
Instrumental Support			
Willing to give time and facilities	Never	1	3
	Occasional	4	12.1
	Frequently	2	6.1
	Always	26	78.8
Take an active role in the treatment	Occasional	3	9.1
	Frequently	2	6.1
	Always	28	84.8
Willing to pay for treatment	Never	1	3
	Occasional	2	6.1
	Frequently	5	15.2
	Always	25	75.8
Meet the required needs	Never	1	3
	Occasional	2	6.1
	Frequently	4	12.1
	Always	26	78.8
Information Support			
Inform patient’s medical diagnose	Never	20	60.6
	Occasional	7	21.2
	Always	6	18.2
Remind patient on meals	Occasional	2	6.1
	Frequently	4	12.1
	Always	27	81.8
Provide information about patient’s health conditions	Never	1	3
	Occasional	3	9.1
	Frequently	7	21.2
	Always	22	66.7
Answer questions from patient related to it’s disease	Never	3	9.1
	Occasional	4	12.1
	Frequently	8	24.2
	Always	18	54.2
Award Support			
Praise the patient when follow doctor’s orders	Occasional	5	15.2
	Frequently	11	33.3
	Always	17	51.5

Characteristic	Frequency	n	%
Support patients during treatment	Frequently	30	90.9
	Always	3	9.1
Cheers the patient if feel sad	Occasional	2	6.1
	Frequently	8	24.2
	Always	23	69.7
Total Score Family Support			
Good		25	75.8%
Adequate		8	24.2%

Table 2. showed the majority of family support given to cancer survivors was in a good category (75.8%). The majority of cancer survivors' companions always provide support during care and treatment, such as reminds to meal, giving time, providing information, and giving appreciation.

Overview of Consumption Patterns of Cancer and Non-Cancer

Table 3. showed that non-cancer subjects (69.7%) significantly consumed more starchy food than cancer subjects (15.2%). Both groups consumed cereals and nuts/seeds. The majority who consumed all dairy products (6.1%) and animal groups (57.6%) were non-cancer subjects. In food ingredients, eggs are consumed the most were cancer subjects (90.9%). In the consumption of green vegetables, there was a significant difference between cancer subjects (57.6%) and non-cancer subjects (87.9%). The most cancer subjects are not comsumption green vegetables and choose other vegetables such as carrot, eggplant, broccoli, cauliflower, mushrooms, and tomatoes. Vegetables and fruit containing sources of vitamin A were mostly consumed by non-cancer subjects (54.5%) while cancer subjects consumed a lot of other vegetables and fruits (52.5%).

Table 3. Overview of Consumption Patterns of Cancer and Non-Cancer Subjects

Variabel	Consumption	Category		
		Cancer	Non-Cancer	p-value
All starchy staples	Yes	5 (15.2%)	23 (69.7%)	<0.001*
	No	28 (84.8%)	10 (30.3%)	
Cereals based food and peas	Yes	33 (100%)	33 (100%)	-
	No	0 (0%)	0 (0%)	
Nuts	Yes	33 (100%)	33 (100%)	-
	No	0 (0%)	0 (0%)	
All dairy milk	Yes	0 (0%)	2 (6.1%)	0.151
	No	33 (100%)	31 (93.9%)	
Meat	Yes	12 (36.4%)	19 (57.6%)	0.084
	No	21 (63.6%)	14 (42.4%)	
Eggs	Yes	30 (90.9%)	29 (87.9%)	0.689
	No	3 (9.1%)	4 (12.1%)	
Green Leafy Vegetables	Yes	19 (57.6%)	29 (87.9%)	0.006*
	No	14 (42.4%)	4 (12.1%)	
Vegetables and vitamin A rich food	Yes	16 (48.5%)	18 (54.5%)	0.622
	No	17 (51.5%)	15 (45.5%)	
Others vegetables and fruit	Yes	17 (51.5%)	14 (42.4%)	0.459
	No	14 (48.5%)	18 (57.6%)	

Yes = 1; No = 0

* Significant (p < 0,05); ¥ Chi square

Differences in IDDS Scores for Cancer and Non-Cancer Subjects

Table 4. showed the average consumption pattern in cancer and non-cancer subjects was significantly different with the average in cancer subjects was lower (4.27±1.13) compared to non-cancer subjects (5.64±1.58). In thus, that cancer survivors have a low diversity of food compared to non-cancer.

Table 4. Differences in Consumption Patterns of Cancer and Non-Cancer

Variable	Cancer	Non-Cancer	p-value
Consumption Patterns	4.27±1,13	5.64±1,58	<0.001*

DISCUSSION

The results showed a significant difference in consumption patterns between cancer survivors and non-cancer with $p<0.001$ (Table 4). Looking at the consumption patterns scores in each subject using the Individual Dietary Diversity Score (IDDS) form, this form was able to picture the quality of diet and the diversity of food consumed by individuals. IDDS has 9 food groups consisting of starchy staple foods, cereals or peas, legumes and seeds, all dairy products, animals, eggs, green leafy vegetables, vegetables or fruit sources of vitamin A, as well as other vegetables and fruits.[22]

Diversity of foods taken using FFQ then converted into the IDDS questionnaire. The advantage of using FFQ is the ability to see the frequency of subjects in consuming foodstuffs since it is more specific particularly the intake per day, week, and month. In addition, intake data retrieval at a one-time makes this FFQ is more effective and suitable for cancer subjects in shelter houses. During the pandemic condition, subjects were more restricted by the rules which can only visit shelter houses within 1-2 days, making FFQ more ideal to use compared to 1x24 hr for 3 days. However recall 1x24 hr for 3 days has the advantage of being able to see the variation and number of servings converted to find out the average of each serving eaten in non-consecutive 3 days.

In this research, the cancer subjects taken were chemotherapy patients since the treatment or therapy had side effects such as nausea and vomiting. Chemotherapy-induced nausea and vomiting (CINV) affect the daily lives of cancer survivors. In the study of cancer survivors who experienced nausea and vomiting about 90% will decreased appetite. This has occurred since anti-tumor substances in chemotherapy affects the hypothalamus and the brain's chemo-receptors to experience nausea and vomiting, thus can affect the intake of food and fluids in survivors undergoing chemotherapy. [3,4,23]

The majority of cancer survivors in this shelter house experienced severe nausea and vomiting. Therefore, these subjects consumed meals which not stimulate their symptoms such as green bean porridge, boiled eggs, and spinach. In addition, nausea and vomiting were felt in cancer survivors within 1 to 3 days post-chemotherapy. Cancer subjects also limited their food intake and consumed in small amounts. If these subjects did not felt nausea and vomiting, then cancer survivors ate in normal portions of food on the 4th day and so on. Research on East Java about the eating habits of cancer patients after chemotherapy, the acceptable foods were rice, eggs, tempeh, tofu and kale.[25]

The age range of the subjects used in the study was 20 to 59 years which is the range of age with the highest prevalence of cancer incidence. The older the age, there will be an increasingly vulnerable the immune system and a decrease in cell function. This usually happens at the age of 50 and above.[24]

Table 2. showed that family support in this study was mostly good, particularly items that affect the consumption patterns of cancer survivors. This, including in instrumental and informational support, the majority of families support well in motivating cancer survivors in increasing appetite. Instrumental support is provided to cancer survivors in the form of meeting the needs of nutritious food and patient facilities. In contrast to the support of information in the form of reminding patients to consume meals and beverages, and also providing information about recommended and not recommended foods.[13,25]

Consumption patterns were influenced by socioeconomic level, nutritional knowledge and access to food availability. Theoretically, research focused on consumption patterns in subjects with low-economy levels showed that dietary diversity had a lower score due to limited access to food.[10,11] However, the study confirmed that cancer survivors and families did not have difficulties in obtaining food. This showed the majority of subjects had a diverse diet and were easy to access. Foods consumed in this subject such as rice, vegetables, meat, eggs, tofu, and tempeh as side dishes. In contrast to subjects who find it difficult to access food because they can only eat foods such as rice, vegetables, eggs, tempeh as side dishes. Access to food availability for both cancer and non-cancer subjects has similarities 64% easy to access food.

Research on cancer survivors who experience nausea and vomiting conducted in Malaysia showed that these subjects had difficulty in eating food adequately thus only consumed a small amount of food such as bread and biscuits. The study conducted in Kenya, cancer survivors who experienced nausea vomiting and including low economic level showed that cereals and nuts were the two most frequent food they consume since it is easier to digest by cancer survivors compared to meat and eggs. Meat and eggs were the least to be consumed because of the high price and tend to cause nausea and vomiting. In addition, milk and other dairy products also increased nausea and vomiting since it was high-fat ingredients.[7,8,26] Both subjects of this study rarely consumed milk since it is difficult to access when it comes to the high prices. In addition, cancer subjects in this study experienced nausea and vomiting when consuming milk and other dairy products.

Results in Table 3. showed that there was a a significant difference ($p<0.001$) between groups in starchy food consumption. Cancer subjects (15%) consumed less starchy food compared to noncancer. Although starchy food was beneficial for increasing the calories they need, they prefer not to consume it because of the

feeling of nauseous and experience vomiting. Snacks for example bread, biscuits, and traditional snacks were only consumed when cancer survivors are waiting in line for therapy. This condition were pictured by minimal consumption for the past month based on FFQ analysis.[27] Meanwhile, the majority of non-cancer subjects (69.7%) chose to eat starchy foods. Most of them were deeply fried, such as fermented soy tempura, vegetables tempura, and fried bananas.

When compares to starchy food, cereals were the most favourable carbohydrates-based food in both groups. Basically, this group of food, particularly rice is a staple food for families in developing countries, including in Indonesia. Carbohydrates are useful for preventing excessive body protein breakdown, mineral loss, and helping fat and protein metabolism. In addition, cancer survivors need a high energy intake to carry out chemotherapy so that the body is well-prepared and does not become susceptible to infection.[28,29]

In this study, the majority of the both groups consumed legumes because it contained high protein, for example green beans and soybeans. High protein consumption for cancer survivors can reduce nausea and vomiting experienced by cancer survivors.[30–33] Based on food intake data for both cancer and non-cancer subjects, tofu and tempeh were the most commonly consumed food ingredients in a daily basis. Not only it is accessible in terms of its affordable price and is the most popular plant-based food in Indonesia.[34]

In the animal protein group, there was not a significant difference ($p = 0.069$) between cancer subjects (36.4%) and non-cancer subjects (57.6%). The majority of these both subjects only consumed animal protein such as chicken and fish and rarely consumed red meat since the price was not affordable. Previous research studied the relationship between food and digestive tract effects showed cancer survivors rarely eat red meat because it is rich in fat, which tends to increase nausea and vomiting. Other research on cancer subjects in India stated that excessive fat consumption can increase the risk of cancer, because fat has cancer promoting properties. Food rich in fat leads the body to produce more estrogen and abnormal cell division. Thus cancer survivors need to limit red meat and preserved products.[26,35–37]

Furthermore, the majority of both subjects often consumed eggs ($p = 0.500$) because it is more affordable and accessible than red meat. In addition, cancer survivors also prefer to eggs that do not trigger nausea and vomiting and are more well received. The egg was packed with high nutritional content since its protein can induce apoptosis in cancer cells, protect against DNA damage, reduce the invasiveness of cancer cells, and exhibit cytotoxic and antimutagenic activity in various cancer cell lines. Eggs are a highly nutritious food source and essential amino acids. This compotition needed by the body for the healing process replace damaged tissue, and form body defense system. The protein contained in egg white and egg yolk are considered as functional food substances because it has biological activities such as antimicrobial, antioxidant, anticancer, and immunomodulatory activities.[38]

A significant difference was shown in terms of green leafy vegetable consumption between groups ($p<0.006$). Most non-cancer subjects consumed more green leafy vegetables, vegetables and fruits containing vitamin A compared to cancer subjects. Based on FFQ analysis, green leafy vegetables that were consumed quite often in these subjects such as spinach, kale, cassava leaves, and papaya leaves. While for vegetables and fruits that contain vitamin A such as carrots, pumpkin, sweet potatoes, mango, and papaya. Other vegetables and fruits that was often consumed such as mushrooms, bean sprouts, beans, long beans, and broccoli. Previous research on cancer chemotherapy subjects, explained that vegetables and fruits can provide protection for breast cancer prognosis since it contained high fiber, vitamins A, C, E, folate, and carotenoids. Vitamin C contains antioxidants that are useful for neutralizing free radicals in the development of cancer. In addition, it also play a role in stimulating the immune system (immunity) and preventing platelet clumping. Consumption of fruits and vegetables can be a good diet and lifestyle. Therefore, vegetables and fruits can be reached from low and high economies.[39] In this study, cancer subjects were still minimal in consuming vegetables and fruits containing vitamin A because the majority chose to consume as desired.

The results of this study showed that the mean score of the consumption pattern of cancer subjects was lower (4.27 ± 1.13) than non-cancer subjects (5.64 ± 1.58). Food groups that were not consumed by cancer survivors according to FFQ analysis were milk, red meat, vegetables and fruits. Not only because of nausea and vomiting experienced by these subjects, but also the ability to access these kind of food. In contrast, non-cancer subjects did not consumed milk and fruits. Therefore, it is necessary to educate both subjects to increase the variety of food and convey the benefits of each food ingredient.. Knowledge of dietary patterns is important since it can provide an understanding of how to fulfill nutrients optimally for each individual.

CONCLUSION

There was a significant difference in consumption pattern scores on cancer and non-cancer survivors who have the same socio-economic background and access to food availability. The average score of the consumption pattern of cancer subjects was lower than that of non-cancer subjects. The food diversity of cancer

subjects was lower than non-cancer subjects. In terms of IDDS food group, starchy foods and green vegetables were two food groups that had a significant difference among cancer subjects and non-cancer.

SUGGESTION

It is necessary to conduct further research by analyzing the diversity of food of each subject using a 3x24 hour recall, food access questionnaire and food security in cancer subjects who experienced CINV compared to non-cancer subjects due to the lack of research in this study field.

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Body Mass Index is The Most Associated Anthropometry Indicators of Obesity with Insulin Resistance in Female College Students

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ABSTRACT

Background: Dysfunction of body tissues due to excessive food consumption is often referred to obesity. Excess storage of visceral fat can develop insulin resistance. Insulin resistance is associated with cardiovascular diseases. Anthropometric measurements can illustrate the early risk of insulin resistance. The aim of this study is to identify the association between anthropometric indicators and insulin resistance.

Materials and Methods: The participants in this study were 163 female students aged 19-24 years who live in Semarang. This is a cross sectional study with a purposive sampling method using the "google form". Anthropometric data that were collected in this study include weight, height, waist cirrcumference, hip, sagittal abdominal diameter. Biochemical data that were collected include blood sugar and insulin levels. The data were analyzed using Pearson Correlation test and Multiple Linear Regression test.

Results: Anthropometric indicators with high risk were 72.4% for Waist to Height Ratio (WHtR); 22.1% for Waist Hip Ratio (WHR); 35.6% for Body Mass Index (BMI); 12.2% for Sagittal Abdominal Diameter (SAD) and 55.2% for waist cirrcumference. Meanwhile, subjects with high Fasting Blood Glucose levels was 16.6%, subjects had the Conicity Index (C-Index) at risk was 74.8% and based on the Relative Fat Mass (RFM) it was 23.9% of the participants were at risk of obesity and high Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) levels were 74.2%. Anthropometric indicators of obesity, including Conicity Index, Relative Fat Mass, WHtR, WHR, BMI, SAD, and waist and hip ratio were all positively associated with insulin resistance. Therefore, multivariate analysis showed that an increase in body mass index is an indicator that is most associated with the insulin resistance ($p<0,001$).

Conclusion: Body Mass Index is the anthropometric indicator that is most associated with insulin resistance.

Keyword: waist-to-height ratio, waist hip ratio, body mass index, sagittal abdominal diameter, conicity index; relative fat mass; insulin resistance

BACKGROUND

Obesity prevalence continues to increase both in Indonesia and globally. Obesity occurs because of an energy imbalance caused by eating food that exceeds the body's function requirements. Physiologically, obesity is defined as a state of abnormal or excess fat accumulation in the tissue.[1] Based on Basic Health Survey (Riskesdas), the prevalence of obesity increased at >18 years of age from 14.8% in 2013 to 21.8% in 2018. Meanwhile, the prevalence of abdominal obesity at >15 years of age has increased by 26.6% in 2013 and 31% in 2018. The indicator used for abdominal obesity is the abdominal circumference with measurements of more than 80 cm for women and more than 90 cm for men [2,3] Meanwhile, the prevalence of obesity in Semarang on people >15 years old was 2.66% in 2018.

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Obesity is one of the factors that is closely related to the development of insulin resistance and type 2 diabetes mellitus.[5] A study in Semarang stated that the storage of visceral or abdominal fat is an implication of insulin resistance, cardiovascular, and other metabolic conditions associated with diabetes mellitus. Excess visceral fat is associated with an impaired insulin sensitivity.[6] A woman tends to be more at risk of fat accumulation in the abdominal and subcutaneous areas associated with insulin sensitivity.[7]

Insulin resistance is a risk factor for metabolic disorders such as impaired glucose tolerance, Non-Insulin Independent Diabetes Mellitus (NIDDM), hypertension, and dyslipidemia. Insulin resistance is when cells in your muscles, fat, and liver don't respond well to insulin and can't use glucose from your blood for energy. To make up for it, your pancreas makes more insulin. Over time, your blood sugar levels go up. Insulin resistance syndrome includes a group of problems like obesity, high blood pressure, high cholesterol, and type 2 diabetes.[8] Insulin resistance is a condition when a person experiences weight gain, especially in the subcutaneous fat tissue and there is accumulation of fat in the stomach, liver, muscles, and further conditions in the brain, arteries and intestines. Most of the fat is stored in the stomach between the organs. This visceral fat develops, contrary to subcutaneous fat, into an active endocrine organ. The adipocytes secrete an abundance of adipokines, which alter the metabolism. Insulin resistance may causes hypertension and increased insulin levels, which can lead to cardiovascular diseases and type 2 diabetes mellitus.[8] Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) is an efficient instrument used by most of the population to identify insulin resistance already in the pre-diabetes stage.[9] Research in 2014 stated that Asian-Indian individuals have higher insulin resistance compared to Europe which leads to type 2 diabetes mellitus. Insulin resistance reflects an obesity trend. The study also explained that hyperglycemia in Southeast Asian and Indian individuals showed a response 2-3 times higher than those in Europe.[10]

Obesity in a person can be measured using several indicators or parameters, including Waist to Height Ratio (WHtR), Waist to Hip Ratio (WHR), Body Mass Index (BMI), Sagittal Abdominal Diameter (SAG), and Waist circumference (WC). Waist to Height Ratio (WHtR) or the ratio of waist to body height is measured as one of the indicators of abdominal obesity and is considered to be more accurate than BMI.[11,12] WHtR was first used in the 1990s to detects abdominal obesity and the risk of obesity-related disease. Recent meta-analysis revealed that WHtR is a better indicator to detect hyperinsulinemia, diabetes, hypertension, dyslipidemia, metabolic syndrome, and other health problems. In addition, WHtR is rated as a sensitive, inexpensive and easy-to-use method for insulin resistance screening. Research conducted in Brazil explained that there is a strong relationship between WHtR and HOMA-IR.[13]

Body Mass Index (BMI) is an indicator for determining obesity by dividing body weight in kilograms by height (squared in meters). The World Health Organization (WHO) defines obesity as a person who has a BMI >30 kg/m^2 and a BMI of 25-30 kg/m^2 for overweight. The American Diabetes Association (ADA) recommends that diabetes assessment should be considered for all Asian-American adults with a BMI score of 23 kg/m^2.[5] The use of BMI has a weakness, as it cannot assess the distribution of fat in the body so that it is less sensitive to determine abdominal obesity. The results of a study in Japan indicated that the BMI cut-off point to predict insulin resistance in all populations was 23.5 kg/m^2. Meanwhile the cut-off point for non-diabetic people is 22.7 kg/m^2 and for diabetic people is 23.6 kg/m^2. This shows that a BMI >23 kg/m^2 is a risk factor for insulin resistance and diabetes mellitus.[5]

Sagittal Abdominal Diameter (SAD) is a measurement of the diameter of the abdomen in the sagittal or median plane, also known as the height of the abdomen. SAD is used to measure visceral fat by the subject lying in a supine position so that the subcutaneous fat is pointed to the side and the harder, stiffer visceral fat remains in place so that it can be measured using a caliper. SAD measurement is considered valid and is not influenced by body size. The US study explained that there was no significant difference between SAD, waist circumference, and BMI. However, SAD is the best anthropometric measurement of glucose metabolism compared to other anthropometric measurements when using Oral Glucose Tolerance Test (OGTT), Fasting Blood Glucose (FGB),

HbA1c, and HOMA-IR. Fasting Blood Glucose (FGB), HbA1c, and HOMA-IR. SAD is also the best anthropometric measurement for all sex, BMI, race and age.[14]

The waist-to-hip ratio is calculated by dividing the circumference of the waist divided by the circumference of the hip. Waist circumference measurement can be used to assess metabolic disorders and diagnose insulin resistance. This measurement is more sensitive to assess body fat distribution, particularly on the abdominal wall and can be used to identify the type of fat distribution. A person with a history of insulin resistance may has difficulty losing weight on a diet with a normal carbohydrate percentage. WHO defines a value of >0.90 in men and >0.85 in women as an indicator of the metabolic syndrome.[8,15]

Waist circumference can be used to measure subcutaneous and intra-abdominal fat tissue, additionally, waist circumference has a better correlation with visceral fat mass and is easy to interpret. Visceral fat tissue is closely associated with metabolic complications such as insulin resistance syndrome. Waist circumference has a stronger correlation with total body fat tissue as assessed by BMI compared to waist-to-hip ratio.[16]

Apart from these indicators, the Conicity Index (C-Index) was also found as an anthropometric indicator related to detect the obesity and the distribution of body fat. The C-Index is determined based on the measurements of body weight, height, waist circumference which are indicators of abdominal obesity. Several studies have shown the relationship between the C-Index and increased body fat in adolescent group. In addition, the C-Index is also associated with an increased risk of metabolic diseases, such as hypertension, dyslipidemia, and diabetes mellitus. However, the correlation between C-Index and insulin resistance in female students has not been widely investigated, especially in Indonesia.[17]

Another estimator which is also a simple anthropometric indicator that can be used to predict body fat percentage and has not been widely studied is Relative Fat Mass (RFM).[18] The relative fat mass equation was derived based on data from the National Health and Nutrition Examination Survey (NHANES) 1999-2004 (n = 12,581) and validated against the data from the 2005-2006 NHANES data (n = 3,456).[19] The RFM formula is obtained after analysis on 365 anthropometric indexes. The results showed that RFM predicted total body fat percentage correctly, measured by dual energy X-ray absorptiometry (DXA), among women and men. RFM shows a higher accuracy than BMI and has fewer false-negative cases of body fat-induced obesity among women and men.[20] Woolcott and Bergman (2020) determined the RFM cut off point for as 40% and 30% for women and men, respectively, diagnose obesity and a higher risk of death. RFM was more accurate than BMI to estimate whole-body fat percentage among women and men and improved body fat-defned obesity misclassifcation among American adult individuals of Mexican, European or African ethnicity. Tus, accurate estimation of body fat percentage is highly relevant from a clinical and public health perspective, an aspect that has been endorsed by the American Heart Association Obesity Committee.[21]

Based on these explanations, this study aims to analyze the association between Waist Height to Ratio (WHtR), Waist Hip Ratio (WHR), Body Mass Index (BMI), Sagittal Abdominal Diameter (SAG), Waist circumference (WC), Conicity Index (C-Index) and Relative Fat Mass (RFM) with Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) in female students.

MATERIALS AND METHODS

This is a cross sectional study with a total of 163 participants. The participants were female students aged 19-24 years who live in Semarang between May-July 2020. The data collection was carried out in collaboration with the Cito Banyumanik Laboratory, Semarang, Central Java. This study has received an Ethical Clearance from the Medical / Health Research Bioethics Commission, Faculty of Medicine, Sultan Agung Islamic University Semarang No. 296/IX/2020/Komisi Bioetik.

The target population in this study were all students in Central Java and the reachable population was students in Semarang. The determination of the sample size is based on the sample size calculation formula for the ratio scale in the cross-sectional study plus 10% of the total sample to anticipate drop outs. The total number of subjects was 163 students who were willing to have their

blood tested. Samples were selected based on inclusion criteria, namely willingness to be research subjects, female students in the city of Semarang, aged 19-24, did not consume alcohol, never smoke and were not pregnant. The subject search process starts from the researcher providing information about the research and the subject opportunities needed through flyers or announcements that can be shared via social media, then people who are interested in being the subject are asked to fill out a google form, which will later be selected according to the inclusion criteria. The sampling technique used was a purposive sampling using "google form" because this research was taken while Indonesia, especially Semarang city, was still under the Large-Scale Social Restrictions due to the COVID-19 pandemic.[16]

The independent variables in this study were Waist Circumference, Hip Circumference, Waist-to-Height Ratio (WHtR), Waist Hip Ratio (WHR), Body Mass Index (BMI), Sagital Abdominal Diameter (SAD), and waist circumference with cut-off points adjusted for Asian participants, namely the cut-off point for the waist circumference of women is > 80 cm.[6] Cut off point for ≥0,50 WHtR,[21] ≥0,85 for WHR,[16] >19,3 cm for SAD,[6] and nutritional status using BMI with the normal cut-off point (18,5 – 22,9 kg/m^2) and obesity (≥25,0 kg/m^2) .[22]

The WHtR measurement required body height to be measured using a *microtoise* with an accuracy of 0.1 cm and a waist circumference measured using a Medline with the accuracy of 0,1 cm. WHR measurement used waist circumference and hip circumference using a Medline. Measurement of body weight using digital scales with an accuracy of 0.1 kg. While the measurement of SAD uses an abdominal caliper with an accuracy of 1 mm, the participants had to be in a sleeping position on a straight surface with their legs forming an angle of 90^o, the feet were above the base. The hands were crossed over the chest and measured in a straight line of the hipbone (iliac crest). The subject inhaled and exhaled slowly and held their breath for a moment while they were measured by lowering the caliper arm on the surface of the abdomen just above the navel.[14]

Relative Fat Mass (RFM) was calculated using the equation for RFM = 64 - (20 x Height in meters / waist in meters) + (12 x S),[20] where S = 0 (men), S = 1 (women). Woolcott and Bergman (2020) determined the RFM cut off point for as 40% and 30% for women and men, respectively, diagnose obesity and a higher risk of death.[21] While the Conicity Index (C-Index) was calculated by the equation of waist circumference in meters / (0.109 x (weight in Kg / height in meters) x 0.5). [23,24] Meanwhile, the dependent variable was insulin resistance measured using fasting insulin and fasting blood glucose levels which was converted into HOMA-IR models. HOMA-IR calculated by multiplying fasting insulin (mol / L) by fasting blood glucose level (mmol / L) which is then divided by 22.5. The Cut off the normal value of HOMA-IR in adolescent was <1.65.[6]

Statistical analysis was performed using SPSS software version 22 to describe the characteristics of the subject and the variables investigated. A correlation test using Pearson Correlation was performed to see the association of each anthropometric indicator with insulin resistance. After the multiple linear regression test conditions are met, then multivariate analysis can be performed using followed by Linear Regression test to determine which anthropometric indicators are the most associated with insulin resistance.

RESULTS

Tables 1 and 2 presented the characteristics of the participants and the frequency distribution of studies conducted on female students in Semarang to determine the anthropometric indicators that correlated with insulin resistance.

Table 1. Subjects Characteristics

Variable	Minimun	Maximum	Mean	Mean ± SD
Waist Circumference (cm)	58,00	112,10	79,44	10,78
Hip Circumference (cm)	80,60	138,45	98,96	9,30
Waist Height Ratio (WHtR)	0,37	0,71	0,51	0,07
Waist Hip Ratio (WHR)	0,67	0,96	0,80	0,06
Conicity-Index (C index)	0,99	1,78	1,19	0,95
Relative Fatt Mass (RFM)	22,02	47,81	35,89	5,52
Body Mass Index (kg/m^2)	15,81	39,30	24,04	4,72
Sagital Abdominal Diameter (cm)	11,35	25,50	16,79	2,42

Variable	Minimun	Maximum	Mean	Mean ± SD
Fasting Blood Glucose Levels (mg/dL)	66,00	110,00	92,00	7,59
Insulin Levels (µg/mL)	0,84	173,00	12,28	13,87
HOMA-IR (ratio)	0,17	8,34	2,58	1,41

The mean value of the BMI was 24.04 kg/m^2, and there was a participant who had a BMI of 39.3 kg/m^2. The average waist circumference of the subjects was 79.44 cm, and it was lower than the average hip circumference, which reached 98.96 cm. Meanwhile, the mean fasting blood glucose level was 92 mg/dL, the mean insulin level was 12.28 µg/mL and the mean HOMA-IR level was 2.58.

Table 2. Subject Characteristics based on Anthropometric Indicators Waist Circumference WHtR, WHR, IMT, SAD, Fasting Blood Glucose Levels, Insulin Resistance

variables	n	%
C-Index		
Normal (>1,14)	41	25,2
At Risk (≤ 1,14)	122	74,8
Relative Fat Mass (RFM)		
Non Obese <40%	124	76,1
Obese (Risk)≥40%	39	23,9
WHtR		
Normal (<0,50)	45	27,6
Risk (≥0,50)	118	72,4
WHR		
Normal (<0,85)	127	77,9
Central Obesity (≥0,85)	36	22,1
Body Mass Index		
Underweight (< 18,5 kg/m^2)	6	3,7
Normal (18,5 – 22,9 kg/m^2)	71	43,6
Overweight (23-24,9 kg/m^2)	28	17,2
Obese (≥25,0 kg/m^2)	58	35,6
Sagital Abdominal Diameter		
Normal (≤19,3 cm)	143	87,7
Risk (>19,3cm)	20	12,3
Waist Circumference		
Normal (<80 cm)	73	44,8
Obese (≤80 cm)	90	55,2
Fasting Blood Glucose Level(FGB)		
Normal (<110 mg/dL)	136	83,4
High (≥110 mg/dL)	27	16,6
HOMA-IR		
Normal (<1,65)	42	25,8
Resistance (≥1,65)	121	74,2

An overview of the frequency distribution based on the anthropometric profile is shown in Table 2. The characteristics of the participants based on the C-Index, RFM, WHtR, WHR, BMI, SAD and The waist circumference displayed that most of the participants, according to the C-Index value, were 74.8% at-risk subjects, while based on the Relative Fat Mass (RFM), 23.9% of subjects were classified as obese. Furthermore, about 72.4% had a WHtR > 0.50 (at risk), whereas based on the WHR value, 22.1% of subjects had abdominal obesity (> 0.85). Subjects with a BMI >25 kg/m^2 or categorized as obese were accounted for 35.6% of the total participants. However, based on the waist circumference, more participants were found to be obese compared to calculation based on BMI, which was about 55.2%. Meanwhile, only 16.6% of subjects had fasting blood glucose levels above normal, it was significantly different from the HOMA-IR value which showed that most subjects had a risk of insulin resistance as much as 74.2%.

The results of statistical analysis in this study are shown in Table 3 and Table 4. Bivariate analysis using the Pearson Correlation test obtained significant results on all anthropometric indicators to insulin resistance with a significance of p <0.001. It shows that the higher Relative Fat Mass, waist circumference, hip circumference, WHtR, WHR, BMI, and SAD, will increase the score

of insulin resistance. However, the C-Index value in this study is not significantly associated with a significance of p <0.001.

Table 3. Correlation Anthropometric Indicators with Insulin Resistance

Variable	HOMA-IR	
	r[a]	*p*
C-Index	0,155	0,048
RFM	0,455	<0,001
WHtR	0,471	<0,001
WHR	0,270	<0,001
BMI	0,548	<0,001
SAD	0,485	<0,001
Waist circumference	0,485	<0,001
Hip Circumference	0,476	<0,001

[a]Pearson Correlation Test

Furthermore, a multivariate analysis was carried out using the Linear Regression test to analyze the most associated anthropometric indicators with insulin resistance. Multivariate analysis was carried out based on the bivariate analysis with independent variabels that have p-value <0.25 and normally distributed. The results showed that the body mass index (BMI) is the anthropometric indicator that was most closely associated to insulin resistance in the participants, whereas about 29,6 % of the increase in insulin resistance was simultaneously influenced by the high BMI value of the participants while the rest was influenced by unobserved variables. The HOMA-IR constant was -1,359 + 0,164 BMI, which states that each additional 1 kg/m^2 increase in BMI value will increase 0,164 HOMA-IR.

Table* 3. *The Most Associated Anthropometry Indicators of Obesity with Insulin Resistance in Students

Variables	HOMA-IR			
	SC[a]	*p*	p-Anova[c]	*Adjusted R Square*
Constant	-1,359			
Body Mass Index	0,164	<0,001[b]	<0,001[c]	0,296[d]

[a]*Unstandardized Coefficient*, [b]Significance, [c]Significance Test F (ANOVA), [d]Coefficient of determination

DISCUSSION

This study was conducted on female students in Semarang, 35.6% of the participants were categorized as obese based on BMI, and 55.2% of participants had abdominal obesity based on waist circumference measurements. Based on the Basic Health Survey (Riskesdas) in 2013, the prevalence of obesity in adults aged ≥18 years reached 14.8% and increased to 21.8% in 2018. Meanwhile, abdominal obesity at ≥15 years of age in 2013 and 2018 reached 26.6% and 31%, respectively.[3]

Obesity in female students is one of the risk factors of insulin resistance. Insulin resistance is a condition that arises due to weight gain, causing an accumulation of fat in the stomach, liver, muscles and at an advanced stage in the brain, arteries and intestines. Most of the fat is stored in the abdomen and between the organs. This visceral fat develops, contrary to subcutaneous fat, into an active endocrine organ. The adipocytes secrete an abundance of adipokines, which alter the metabolism. The major problem that induces insulin resistance is a rise in blood pressure and insulin levels which can lead to cardiovascular diseases and type 2 diabetes mellitus.[8] Furthermore, insulin resistance can go through various mechanisms that can contribute to the accumulation of macrophages in the walls of blood vessels, thereby increasing atherosclerosiss

Insulin resistance can be assessed using the HOMA-IR model from fasting insulin and fasting blood glucose levels. The results of HOMA-IR in this study indicated that 74.2% of subjects showed insulin resistance with a mean value of HOMA-IR >1,65. In line with the previous study from Dieny et al in female students, the prevalence of participants with high levels of HOMA-IR was 83.3%.[6] Bivariate analysis showed that all tested anthropometric indicators related to the levels of HOMA-IR. Followed by the multivariate test, it was found that the BMI had a strong correlation with HOMA-IR levels.

A research conducted on the Japanese population indicated that a person who has a BMI ≥23 has a risk of insulin resistance and diabetes mellitus. American Diabetes Association suggests that for diagnosing Diabetes Mellitus in an individual, BMI ≥23 kg/m^2 should be considered. Moreover, a study from Japan, non-diabetic people without hypertension, hyperglycemia, or dyslipidemia indicated that there is a risk of insulin resistance in Japanese people who have a BMI ≥23 without any cardiometabolic risk factors.[5] In this study, the average BMI of the participants was 24 kg/m^2 and BMI is one of the indicators most closely associated with insulin resistance.

Another indicator in this study that has a positive correlation with insulin resistance are the Waist to Hip Ratio (WHR) and Waist to Height Ratio (WHtR). These two indicators measure the waist circumference which can explain abdominal obesity in a person. Abdominal Obesity caused an increase in corticosteroid and adrenal hormones produced by the adrenal glands and can affect the level of glucose in the blood. Overweight people will have an increased lipids levels in their bodies. One type of lipid tissue is a fatty acid. Peripheral tissues that are exposed to the increase in free fatty acids will induce insulin resistance. Mechanically, the activation of the threonine kinase pathway by free fatty acids metabolism will reduce insulin receptors. Long-term exposure to fatty acids in the pancreas will damage the beta cells, this condition called lipotoxicity. It has an impact on fasting blood glucose which causes WHR and WHtR values as central parameters of obesity associated with insulin resistance and type 2 diabetes.7[25]

Another study reported that anthropometric measurements such as waist circumference, BMI, WHR and WHtR are easy and inexpensive methods in clinical and epidemiological fields.[29] Meanwhile, other studies suggest that WHtR can be a screening tool for markers of insulin resistance in children and adolescents by considering the ratio, costs and benefits.[30,31] Besides, a study conducted in Peru on women over 18 years of age with normal BMI reported that waist circumference and WHtR were associated with a high ratio of triglyceride levels and HDL cholesterol. Whereas triglycerides and HDL cholesterol are components of metabolic syndrome. Insulin resistance plays an important role in the pathophysiology of metabolic syndrome.[26]

Abdominal obesity and diabetes are closely related. However, there are different conclusions regarding the better abdominal obesity index that can predict insulin secretion. A study from China by Liu et al. on obese patients reported that anthropometric measurement of WHR was found to be the most relevant index of obesity at each phase of insulin secretion among obese patients and could be a good predictor of β-cell insulin secretion function.[27] In line with a study by Zapata et al. on female subject of normal weight without type 2 diabetes mellitus, stated that WHR can be an anthropometric indicator for early detection of insulin resistance, including the healthy subjects.[28] In China, a study by Song et al. on children and adolescents participants was using the Mendelian Randomization (MR) method which has been widely used for cardiometabolic observation studies. MR method is a controlled trial where the genotype is randomly distributed at conception, is less likely to be influenced by reverse causation and confounding factors. Single Nucleotide Polymorphisms (SNP) associated with BMI and WHR were selected from the Genome-Wide Association Studies (GWAS). They found that a genetic predisposition to a higher BMI or WHR was associated with altered cardiometabolic traits. When compared with general obesity, abdominal obesity might have stronger effects on glycemic traits and blood lipids.[29]

Abdominal obesity is usually assessed by waist circumference, but recent studies have reported that it can be assessed using the Sagittal Abdominal Diameter (SAD), also known as abdominal height. A study by Moller *et al.* stated that SAD was significantly correlated with several markers of cardiometabolic risk factors other than waist circumference and BMI.[30] While a cohort study in Sweden stated that SAD >25 cm in patients with type 2 DM could be used as an independent risk assessment to identify high risk of cardiovascular disease if confirmed in a larger study.[31] Accumulation of certain fat depots is related to poor metabolic outcomes. Visceral adipose tissue can locally affect the heart and coronary arteries vasocrine or paracrine secretion of proinflammatory cytokines. A study by Vasques et al. on female aged >20 years stated that the amount of stored fat is associated with cardiometabolic risk factors in various kinds of adiposity and tolerance levels. SAD measurements correlated with epicardial adipose tissue (EAT). Furthermore, SAD is strongly

associated with atherogenic lipoprotein subfraction insulin resistance and cardiovascular risk. The metabolic characteristics of EAT may explain the association with cardiometabolic risk parameters. Adiponectin expression was significantly higher in subcutaneous fat, which may explain the poor insulin sensitivity associated with fat tissue accumulation.[32]

One of the anthropometric indicators, Conicity Index (C-Index) is an assessment of the distribution of fat mass at risk of metabolic disease. It can describe the condition of total body fat. The higher the C-index value, the higher a person's risk for metabolic disease.[33] If the C-index value is more than 1.14, it is considered as being at risk for metabolic disease.[18] The results of other studies suggest that the C-index can detect levels of C-reactive protein (CRP) and fibrinogen which are associated with an increased risk of cardiovascular diseases compared to anthropometric indicators of BMI, WC, and WHR.[34] Cardiovascular disease is a complication that causes a lower quality of life and increased morbidity also mortality.[35] Moreover, the C-index is also positively associated with insulin resistance, HT, and dyslipidemia.[36] Nevertheless, according to the present study, C-index is not associated with HOMA-IR (p = 0,048. These results are consistent with a prospective study in China which showed that the predictive power of diabetes from the C-index was still below the Body Roundness Index, waist circumference, and abdominal volume index (AVI).[37]

This variation in results may be due to differences in body fat composition and distribution between ethnic groups. Obesity is considered a major cause of IR in general population. An increased adipose tissue induces a change in the release of free fatty acids and the secretion of adipokines and proinflammatory cytokines, which are involved in the modulation of insulin sensitivity.[38] A woman with a Relative Fat Mass (RFM) >35 is considered at risk of obesity and associated mortality. RFM is more specific than BMI because it considers the differences of gender in body adiposity.[21] In our study, high RFM was significantly associated with an increase in HOMA-IR levels with a 23.9% percentage at risk of obesity. This finding is in line with a study by Karava et al. which showed that RFM level is a risk factor for IR even in normal-weight children and patients with glucose and insulin metabolism disorders.[38] RFM measurements have been reported in other studies to estimate body fat percentage such as the use of dual-energy X-ray absorptiometry (DXA).[39] Another study suggests that using RFM in conjunction with BMI to provide additional information when assessing obesity in women can improve diagnostic accuracy.[40] Anthropometric measurements using RFM can represent obesity diagnostically and thus provide better predictability for a wide range of dyslipidemia (high LDL, low HDL, and high triglycerides) and metabolic syndrome, which are independent risk factors for coronary heart disease, peripheral artery disease, stroke, and death.[41]

Although BMI is widely used to determine weight categories, anthropometric parameters of central obesity are reported to be a better screening tool to identify prediabetes in Asian multiethnic populations.[42]

Likewise with the results of this study. The potential mechanism of central obesity in promoting the development of prediabetes is through the role of visceral fat which has more metabolic activity than subcutaneous fat, namely by secreting nonesterified free fatty acids and proinflammatory adipokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-6 which can be causes insulin resistance and pancreatic beta cell damage, resulting in impaired glucose tolerance and eventually developing diabetes.[43,44]

Women are known to have a higher optimal cut-off BMI than men. This is due to differences in fat storage patterns between sexes, where women have a tendency to store fat in the subcutaneous area, especially in the gluteofemoral location so that it is better for large and long-term storage than men who have a tendency to store fat in the visceral area.[45]

Research shows that visceral fat is more closely associated with cardiometabolic risk in women than men. This suggests that although women have less visceral fat overall than men, the accumulation of visceral fat in women carries a greater risk of developing cardiometabolic disorders.[46]

However, in this study, women actually had an optimal cut-off value of anthropometric parameters markers of central obesity which was higher than men. This is probably because the majority of the male sample in this study were active smokers. Nicotine (the main bioactive

component of cigarette smoke) can activate the sympathetic nervous system leading to increased lipolysis in white adipose tissue thereby increasing the release of free fatty acids that contribute to insulin resistance and weight loss.[47]

Insulin resistance happens to cause a decline of tissue sensitivity to insulin which main function is to stimulate glucose uptake. If obesity resulted in insulin resistance, increased blood glucose and hyperinsulinemia, it will end with the development of type 2 diabetes mellitus and metabolic syndrome.[48] Thereby, anthropometric measurements can be used for screening to control obesity in early life, especially in abdominal obesity, to prevent cardiometabolic dysfunction later in life. However, no single anthropometric indicator is consistently superior in predicting markers of cardiometabolic risk.[30]

CONCLUSION

Insulin resistance can be identified significantly using anthropometric indicators namely Conicity-Index, Relative Fat Mass, waist circumference, hip circumference, WHtR, WHR, BMI, and SAD. However, results indicated that BMI is the anthropometric indicator that was most associated with the HOMA-IR among other indicators. Therefore, it can be used as a fast and efficient anthropometric measurement in identifying the occurrence of insulin resistance in female students.

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