FEASIBILITY STUDY TO TEST ANTI-OBESITY EFFECTS & SAFETY OF LONG-TERM CONSUMPTION OF MOMORDICA CHARANTIA (BITTER MELON)

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Abstract

As the obesity epidemic increases globally, several weight loss interventions are being considered for successful management strategies, including alternative medicine. *Momordica charantia* (bitter melon) is consumed in many Asian countries and has traditionally been used for its medicinal properties. Animal and cell culture studies indicate that MC can prevent diabetes as well as hyperlipidemia and obesity. Several clinical studies have demonstrated hypoglycemic effects of MC. However, a clinical study to test the efficacy of MC on plasma lipids and weight reduction in humans is lacking. The main objectives of this study were to explore the feasibility and safety of long-term consumption of MC and to investigate the effects of MC on body weight and plasma lipid profiles.

Healthy, overweight and obese subjects were recruited and received MC fruit powder for daily consumption without dietary and lifestyle changes. The results showed that many subjects indicated a high level of willingness to consume MC daily in the feasibility study. Also, the feasibility of daily MC consumption is possibly enhanced by additional supplementation that masks the bitterness of MC. The present study faced difficulties in recruiting and retaining eligible subjects for the clinical trial, there was only one subject who qualified and participated in the trial among 25 subjects who were recruited as possible candidates. Thus, efficacy and safety of daily consumption of MC for anti-obesity treatment remain unknown. Further investigations of efficacy and safety of MC on obesity with a large sample of randomized clinical trial design could indicate a possible alternative approach in obesity treatment.

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Abbreviations

ALT	Alanine amino transferase
ASAT	Abdominal subcutaneous adipose tissue
AST	Asparatate amino transferase
BMI	Body Mass Index
CAM	Complementary and Alternative Medicine
CDC	The Center of Disease Control and Prevention
CH	Cholesterol
CHD	Coronary heart disease
CHS	Committee on Human Studies
CVD	Cardiovascular disease
DLSs	the Diagnostic Laboratory Services
DM	Diabetes
FDA	Food and Drug Administration
FFQ	Food Frequency Questionnaires
FPG	Fasting plasma glucose
FV	Fruit and vegetables
GERD	Gastroesophageal reflex
HbA1C	Hemoglobin A1C
HDL	High-density lipoprotein
HFCS	High-fructose corn syrup
HFD	High-fat diet
HNFAS	Human Nutrition, Food and Animal Sciences

IAAT	Intra-abdominal adipose tissue
LDL	Low-density lipoprotein
MC	Momordica charantia
MetS	Metabolic syndrome
NCCAM	the National Center for Complementary and Alternative Medicine
NHANES	National Health and Nutrition Examination Survey
OGTT	Oral Glucose Tolerance Test
SGOT	Serum Glutamic Oxaloacetic Transaminase
SOC	Stages of Change
TGs	Triglycerides
T1D	Type 1 diabetes
T2D	Type 2 diabetes
UHM	University of Hawai‘i at Mānoa
USDA	United States Department of Agriculture
WHO	The World Health Organization

Chapter 1: Obesity

1.1 Definition

Obesity is defined as an accumulation of excess body fat in humans and is a chronic inflammatory disease [1]. The World Health Organization (WHO) defines obesity in adults by determining a person's Body Mass Index (BMI) which is weight in kilograms (kg) of a person divided by square of the person's height in meters (m^2) [2]. BMI is the most widely used method to identify health risks and mortality rates relating to body fat [3]. WHO classifies the BMI range of healthy normal weight as 18.5 to 24.9 kg/m^2, overweight as 25 to 29.9 kg/m^2, and obese as above 30 kg/m^2 [2]. The obese category is subdivided into three classes, class 1 as BMI of 30.0 and 34.9 kg/m^2, class 2 as 35.0 to 39.9 kg/m^2, and beyond 40.0 kg/m^2 as obese class 3 [4]. For children and adolescents aged 2 to 19 years old, the sex-specific BMI-for-age growth charts created by the Center of Disease Control and Prevention (CDC) are used to define childhood obesity. BMI between the 85th and 95th percentiles for the age is classified as overweight, and at or above 95th percentiles as obese [5].

Although BMI highly indicates individual adiposity stores without any bias of height, it should be noted that BMI cannot determine the distribution of adipose tissue in the body, and also a person's body shape [6]. For instance, two individuals who have the same BMI can have different amounts of total body fat, intra-abdominal adipose tissue (IAAT), and abdominal subcutaneous adipose tissues (ASAT). IAAT is more highly correlated with obesity-related health risks than other locations of adipose tissues [7]. Therefore, additional body assessments

including waist circumferences, percentage of total free fat mass and lean body mass also should be measured along with BMI accurately to assess obesity [1].

In addition, BMI, and the amount of body fat differ between ethnic groups. Asian populations generally, have a higher percentage of body fat at a relatively low BMI range compared with non-Hispanic white [6]. Using the same BMI classification as WHO, Japan, China, Vietnam, and India have reported lower rates of obesity compared with other Asian countries. However, the incidence of type 2 diabetes (T2D) and cardiovascular disease (CVD) rise among those countries with BMI less than 25. This is most likely due to genetic components of controlling the storage of the amounts and locations of body fat [8]. Therefore, BMI cut-off points should be adjusted based on ethnicity.

1.2 Obesity statistics

The International Obesity Task Force estimates the prevalence of overweight adults world wide is approximately 1.0 billion people, with an additional 475 million who are obese [9]. This indicates that approximately one in every 14 people is obese. Among the global obesity trend, the prevalence of obesity in the United States has been dramatically increasing for the past 50 years and now has the highest proportion of obese people per capita worldwide [2]. In 1960, 13.4% of the U.S. adults were obese [10]. By the period 2003 to 2004, the prevalence of obesity increased to the range 28.5% to 31.0% depending on the age group [4]. In 2009-2010, 35.5% of adult men and 35.8% of adult women were obese and nearly 70% of adults in the U.S. were either overweight or obese, an almost three fold increase compared to in 1960 [3, 10]. In

addition, the percentage of children with childhood obesity has tripled in the past 30 years in the United States [8]. In the period of 2009-2010, 16.9% of children and adolescents aged 2 to 19 years old are obese and 31.8% children and adolescents were either overweight or obese [5].

Prevalence of obesity is correlated with ethnicity. In the United States, both Hispanics and African Americans have a higher BMI than the non-Hispanic white and Asian populations. According to the CDC, 41.9 % of non-Hispanic black women and 30.7 % of Hispanics are obese which are the highest rates of obesity among ethnic groups [11]. In addition, the latest National Health and Nutrition Examination Survey (NHANES) data collection for children and adolescents reported that 21.2% Hispanics and 24.3% of non-Hispanic blacks were obese while only 14.0% of non-Hispanic whites were obese [5].

In Hawai‘i, the latest self-reported data collected by the CDC in 2011 found that the prevalence of obesity among adults was 21.8 %, the second lowest obesity prevalence state among the fifty states [11]. Among ethnic groups in the state of Hawai‘i, Native Hawaiians, defined as an original of the Hawai‘i islands, have the highest obesity prevalence [12]. The U.S. Department of Health & Human Services reported that 43.5 % of Native Hawaiians/ Pacific islanders [13] are obese in 2010 [14].

1.3 Health complications of obesity

Obesity is strongly linked to numerous health complications which are associated with increased morbidity and mortality [1, 4, 8, 15]. People with excess body fat develop combinations of

chronic diseases, including T2D, insulin resistance, CVD, coronary heart disease (CHD), hypertension, stroke, certain cancers, dyslipidemia, gallbladder disease, sleep apnea, respiratory problems, and gastroesophageal reflex (GERD) [1, 4, 8, 15]. NHANES examined the prevalence of obesity-related complications using data obtained from 1999 to 2000. NHANES found class 3 obese individuals have hypertension at rates nearly 5 times higher than normal weight individuals [4]. Since 1998, obesity is classified as a major risk factor for CVD by the American Heart Association [15]. CVD is linked to elevated blood pressure, higher levels of total serum cholesterol (CH) and low-density lipoprotein (LDL) cholesterol and lower levels of high-density lipoprotein (HDL) cholesterol. CVD also increases incidence of heart failure and stroke [14]. T2D is another serious health problem in world wide that is strongly correlated to excess weight. It is reported that weight gain is a primary cause for T2D development in more than half of T2D populations [9,15]. Metabolic syndrome (MetS) (the definition developed by NCEP ATP III) describes the condition when a person has a combination of three or more risk factors that increase chances of developing chronic disease. These risk factors include central obesity, dyslipidemia, insulin resistance, and hypertension [4, 15]. In addition to the above mentioned major obesity-related health complications, obesity is also a risk factor for rheumatoid arthritis, spondyloarthropathy, fibromyalgia, and chronic fatigue due to its inflammatory nature [15].

Obesity is not only a leading cause of metabolic disorders, but also associated with psychological disorders, such as depression and anxiety symptoms [15, 16]. Simon *et al*. demonstrated that one out of four obese individuals had experienced depression and anxiety disorders, and suggested that it is observed more often in women [16].

In proportion to increased prevalence of childhood obesity, morbidity and premature mortality have risen significantly in obese children. According to Reilly *et al.,* there was a significant association between obesity and overweight children/adolescence and an increased risk of premature death in the U.S. and Western Europe [17]. In addition, the study found strong evidence of a relationship between childhood obesity and onset of diabetes (DM), CHD, hypertension, and stroke in their later life [17].

1.4 Pathogenesis of obesity

Multiple factors that control energy balance are involved in the pathogenesis of obesity [1, 8, 18]. These are genetic, metabolic, social, behavioral, cultural, and physiological factors. Genetic and metabolic factors play important roles of maintaining energy homeostasis, and in regulating energy repletion and depletion by releasing certain hormones and neurotransmitters [18]. The neural signals leptin and ghrelin govern hunger and satiety levels which in turn control food intake ultimately influencing body weight [8]. Disruption of the signals by other factors leads to an energy imbalance resulting in over eating and storage of excess body fat. Genetic influences also contribute to the prevalence of obesity among ethnic groups as well as the relationship between maternal obesity and obesity in their children [8, 19]. For instance, children have a greater risk to be larger for gestational age from obese mothers and are two times more likely to be obese later in life to develop obesity-related conditions such as insulin resistance [19].

In addition to the metabolic and genetic influences, social, behavioral, cultural, and physiological factors also play significant roles in the prevalence of obesity [1, 8]. "Obesogenic" environment is a term used to describe the environment that promotes weight gain and contributes to obesity. This environment contributes to over consumption of foods via easily accessible inexpensive energy-dense foods and large portion sizes [8, 20]. It has been clearly recognized that consumption of high-fructose corn syrup (HFCS), often found in soft drinks and bakery goods, and energy-dense (kcal/g) foods such as fast foods have increased together with the increased incidence of obesity [8]. In addition, the consequence of consuming excess energy dense foods and beverages leads to a lower intake of fruit and vegetables (FV) [21, 22]. Less than one in four (23.4%) individuals in the U.S. meets the daily recommendation of FV consumption [23]. Increasing the consumption of FV along with reducing calorie and fat intakes can help reverse the development of obesity. Whigham *et al.* and some other clinical studies demonstrated that increased consumption of FV was effective in weight and fat loss [24-27]. Furthermore, Ledikwe *et al.* indicated that the U.S. adults who consumed more FV had the lowest energy density diet and the lowest prevalence of obesity [28].

An obesogenic environment not only provides less healthy food choices, but also includes less physical activity and a sedentary lifestyle [8, 20]. Numerous of studies demonstrate that watching television negatively impacts physical activity levels and induces over eating and an increased choice of energy-dense-foods, as well as reduces resting metabolic rate [20]. Socioeconomic status and stress factors are the other two components of obesogenic environments. There is some evidence indicating a correlation in minority groups between lower incomes and less education with increased obesity [1]. Giskes *et al.* found that mothers with

more advanced socioeconomic status had greater intake of FV consumption than those with lesser status [29], suggesting that a higher position in society with a greater salary may help with acquiring more nutrient-dense foods. Psychological stress also can contribute to obesity risk along with socioeconomic factors. Moore *et al.* suggest that individuals who have lower social position had higher stress which was associated with poor dietary behaviors and higher prevalence of overweight and obesity [30]. According to Rudenga *et al.*, stress can promote over-eating and result in weight gain, and these behaviors are seen more often in women and obese individuals compared with lower BMI individuals with similar stress stages [31]. Overall, it is clear that many factors, including gene and environment interaction, promote weight gain, and obesity associated metabolic complications.

1.5 Treatment for obesity

Prevention and management of obesity is crucial for reducing the risks of morbidity and mortality. Evidence demonstrates that weight reduction in overweight and obese individuals reverses the following health risks; 1), lowers blood pressure which reduces the risk of developing hypertension, 2), decreases the risk factors for T2D and CVD, 3), reduces serum triglycerides (TGs), total serum CH and LDL cholesterol, and increases HDL cholesterol, 4), decreases blood glucose levels and reduces prevalence of insulin resistance [1, 4, 15].

Current intervention approaches for obesity management are dietary modifications, increased physical activity, behavior therapy, pharmacotherapy, surgery, and combinations of these approaches [1].

1) Dietary modification:

It is clear that obesity is a result of excess energy intake. Thus, dietary management is a primary strategy for decreasing calorie intake, obtaining and sustaining optimal weight. The 2010 *Dietary Guidelines for Americans* which promotes the most recent evidence-based nutritional guideline, promotes a healthy weight and a reduction of the prevalence of overweight and obesity [32].

There are numerous weight loss programs and strategies available in society, such as low-fat diets, the Dietary Approaches to Stop Hypertension (DASH) diet which emphasizes eating fresh fruit, vegetables, and high quality protein, and low carbohydrates diets such as Atkins and Zone diet [33]. Although some of these programs may be beneficial for a short-term effect, continuous use of weight loss programs for the long-term is unrealistic. Dietary intervention alone has minimal effects in long-term weight reduction. For instance, a randomized, one year controlled trial for diet intervention did not show significance in lipid profiles and body weight changes [34]. Thus, a long-term obesity management strategy of weight-loss and maintenance requires a combination of diet along with other interventions. Also, Baker states that while most people are focusing on diet plans and restricting food items, few people consider portion sizes of foods. Due to the norm of larger portion size in the U.S., he suggests that consumption of smaller portions would help reduce calorie intake and would promote optimal weight loss [33].

2) Physical activity:

Many studies have shown that regular physical activity is effective in weight gain prevention, weight loss, and sustaining weight management [1, 35]. Shiroma *et al.* conducted a prospective cohort study measuring the relationship between physical activity levels and weight management in older men over a period of ten years. They found that men who did an average of 70 minutes of moderate to intensive physical exercise a day maintained their normal weight range, suggesting that regular physical activity for specific amounts of time is effective for weight gain prevention, possibly contributing to weight loss as well [35]. Current recommendation for physical activity levels, provided by the 2008 Physical Activity Guideline for Americans, suggests at least 150 minutes of moderate physical activity per week, also 2 or more days of muscle-strengthening exercises each week for adults 18 to 64 years old, and 60 minutes or more per day for children and adolescent 6 to 17 years old [36]. Although physical activity alone may not be as effective as some other interventions, the combination of physical activity with other strategies can be effective for managing weight.

3) Behavior therapy:

Several behavioral approaches have been associated with success in weight loss and/or management, including self-monitoring [1, 37], stress management strategies [1], social support [1], and motivational interviewing [38]. Akers *et al.* found that daily self-monitoring of weight, combined with physical activity and FV consumption was effective in weight management [37]. Armstrong *et al.'s* study found motivational interviewing, a patient-centered dietary counseling strategy, improved the quality of weight loss and management in overweight and obese

individuals [38]. In addition, Reyes *et al.* found that individuals who successfully maintain their weight loss have positive attitudes and possess highly motivated skills for weight loss management [39]. These findings suggest that behavioral changes can enhance the effects of other interventions in combating obesity.

4) Pharmacotherapy:

Several weight loss drugs have been approved by Food and Drug Administration (FDA) and are available for use by obese adults. These include Phentermine and Diethylpropion, and Lorcaserin, Orlistat, and the combination of Phentermine and Topiramate, available since 2012 [15, 40]. In the case of certain obese populations who have a rare obese-induced genetic component, for example leptin deficiency, recombinant leptin therapy is helpful to aid obesity intervention [40]. Obese individuals may combine pharmacotherapy along with other interventions such as dietary modification and regular physical activity. Adverse effects from use of such drugs have been observed in patients. These adverse effects include agitation, insomnia, headache, tachycardia, hypertension, anorexia, and even sudden death [15, 40]. Understandingly, no pharmacotherapy with guarantees of safety and without side effects exists for both obese children and obese adults [15, 40].

5) Weight loss surgery:

Recently, bariatric surgery has become a popular weight loss strategy, resulting in positive outcomes. The three types of bariatric surgeries have been performed for extreme obesity

include: 1) gastric banding and vertical banded gastropalasty which reduces food intake, 2) long-limb gastric bypass, biliopancreatic diversion, and biliopancreatic diversion with a duodenal switch which decreases absorption of nutrients, and 3) roux-en-Y gastric bypass which is both food restrictive and nutrient malabsorptive [41]. Several studies have found evidence that there is a decreased risk of mortality for obese individuals with health complications who underwent bariatric surgery and lost weight compared with morbidly obese individuals without the surgery [4]. On the other hand, many of patients who have undergone bariatric surgery develop gastrointestinal complications. These complications are vitamin and mineral deficiencies, vomiting, infection, dumping, staple line failure, stenosis, ulceration, bleeding, splenic injury, and death. In addition, the long term success and safety of bariatric surgery is questionable due to insufficient knowledge and evidences of beneficial results with complications [41]. More investigation of the long-term success of bariatric surgery is suggested before use as a weight loss strategy.

Prevalence of obesity is globally widespread and has become a major risk factor for numerous chronic diseases and mortality. Many obesity management strategies are widely applied for intervention, yet successful weight loss and weight regain prevention is still a challenge. At the same time, the population in the U.S., which holds the highest prevalence of obesity world wide, has become more interested in different health care approaches such as traditional or Eastern medicines to seek strategies for anti-obesity intervention.

Chapter 2: Complementary and Alternative Medicine (CAM)

2.1 Introduction

Complementary and Alternative Medicine (CAM) has been recognized and has received interest by the medical field because of growing usage among people with chronic diseases including T2D, MetS, and weight control. An increase in the prevalence of obesity despite a wide variety of obesity treatments has also generated interest in functional foods such as fruits, vegetables, and whole grain products [42-45]. The survey of CAM use in the U.S. in 2007 reports that 38% of adults and nearly 12% of children have used CAM [46]. Another latest survey in 2011 showed that 53% of 50 and older individuals have used CAM with functional foods, herbs, or dietary supplements as the most commonly used forms [47]. The CDC reported that one half of the U.S. population used dietary supplements in 2003-2006 [48]. Adults spent approximately $34 billion on CAM treatment for the past 12 months. Several insurance companies have started offering coverage of some CAM treatments in the last few decades [49].

The National Center for Complementary and Alternative Medicine (NCCAM), led by the U.S. Federal Government for scientific research on CAM, defines CAM as "a group of diverse medical and health care systems, practices, and products that are not generally considered part of conventional/ Western medicine" [50]. Complementary medicine refers to the combined use of CAM and conventional medicine, whereas alternative medicine is the use of CAM as a primary treatment instead of conventional medicine [50]. CAM takes different approaches to treatment, from the use of various herbal medicines, the practice of body and mind integration such as yoga and meditation, to the application of physical treatments including acupuncture and body

massages [50]. Because of the increased recognition of these treatments, the U.S. has offered certification programs in several CAM techniques including acupuncture, Chinese herbology, and oriental body work [49]. CAM has been practiced for a long period of time in many Eastern countries, and the many CAM treatments have demonstrated efficacy for relieving disease conditions in research investigations. A general principle of CAM's focus is that one's health is optimized when there is balance among body, mind, and spirit. When this balance is lost, a disease will enter the body. Thus, CAM treatments approach one's whole body system to regain health rather than just treat a disease symptom [50].

2.2 Ayurveda

Ayurveda or Ayurvedic medicine is a complete system of theory and practice that has been widely practiced in India for more than 5,000 years and is considered as CAM in the U.S. The word Ayurveda is derived from Sanskrit and means "science of life" [51]. Differing from western medicine's principles, Ayurveda has its own unique interpretations of health-science, principles, diagnosis and treatments. In a 2007 survey the number of the U.S. adults who had used Ayurvedic medicine surpassed more than 200,000 people [49].

Ayurveda teaches that a disease or disharmony of health can be reversed with proper diet, correct lifestyle, and rejuvenation. Among these components, proper dietary habits and food choices is the primary means for obtaining health [51]. Many foods and herbs serve as medicines and are

used to treat specific diseases in an Ayurvedic treatment. According to Ayurveda, one of the most useful vegetables to treat diabetes and its complications such as obesity is *Momodica charantia* (MC), commonly known as bitter melon.

Chapter 3: *Momordica Charantia* (MC)

3.1 Background

MC is also known as bitter melon, bitter gourd, balsam pear, bitter apple, or *karela* [52]. MC is a vegetable that belongs to the *Cucurbitaceae* family [53-55], and is widely cultivated in the tropical regions of Asia, Africa, the Caribbean, and South America [53, 54]. MC is a tropical vine with green leaves and yellow flowers [53]. The size of the fruit varies, from 20 to 30 cm for a Chinese phenotype to only 6 to 10 cm for some varieties [56], its shape is long and thin, similar to a cucumber [53].

The fruit of MC is known for its bitter taste, and can be prepared and eaten in many ways such as frying, cooking in curries, drying, pickling, or canning [52]. Leaves and young stems can be cooked as dishes [52], and also brewed for tea. MC is traditionally consumed in India for many medicinal uses including anti-viral, anti-bacterial, anti-malarial, anti-helminthic, anti-tumor, and anti-diabetic as well as abortifacient and anti-fertility medicine [57]. MC fruit is a good source of vitamin C. The United States Department of Agriculture (USDA) database shows that 100 g of fresh MC fruit contains 84.0 mg of vitamin C, 471 IU of vitamin A, 296 mg of potassium and provides only 17 kcals of energy [58]. The fresh leaf contains 1734 IU of vitamin A, 2.04 mg of iron, and 608 mg of potassium per 100 g [59]. Table 1 provides a summary of the nutritional content of MC fruit and leaves. In addition, MC contains several compounds such as charantin, mormordin, polypeptide- ‘p’, flavonoids, and polyphenol that are believed to contribute pharmacological effects [60].

Table 1. Nutritional composition of raw MC fruit and raw MC leaf. Adapted from USDA Nutrient Data Laboratory., http://ndb.nal.usda.gov/ndb/foods/show/2818 [58], http://ndb.nal.usda.gov/ndb/foods/show/2816 [59].

Nutrients	Composition (per 100.0 g)	
	Raw MC fruit	Raw MC leaf
Water (g)	94.03	89.25
Energy (kcal)	17	30
Protein (g)	1.00	5.30
Total fat (g)	0.17	0.69
Carbohydrate (g)	3.70	3.29
Fiber (g)	2.8	84
Calcium (mg)	19	2.04
Iron (mg)	0.43	99
Phosphorous (mg)	31	608
Potassium (mg)	296	88.0
Vitamin C (mg)	84.0	1734
Vitamin A, IU (IU)	471	89.25

3.2 Cell culture studies of MC

The hypoglycemic and hypolipidemic effects of MC fruit have been investigated in some *in vitro* studies. Sitasawad *et al.* demonstrated that MC juice reduced lipid preoxidation and action of anti-programmed cell death in an isolated islet of diabetic mice [61]. Another *in vitro* study showed extract of MC fruit and seed improved glucose uptake in 3T3-L1 adipocytes [62]. Nerurkar *et al.* has demonstrated the hypolipidemic effects [60] and is the first laboratory to demonstrate that MC juice significantly reduced accumulation of lipid droplets in primary human adipocytes [63].

3.3 Animal studies of MC

A majority of studies have shown that the seeds, fresh and dried fruit, leaf, and fruit of MC have effects on hyperglycemia when taken orally. These effects include decreased blood glucose levels [64-74], decreased blood glucose levels along with an antioxidant action [61, 75], increase plasma insulin concentrations [76], enhanced blood glucose tolerance [77], and an increased in the number of beta-cells [78]. Hypolipidemic and anti-obesity properties of MC have also been documented [74-77, 79-82]. Chen *et al.* demonstrated that MC juice was effective on weight gain prevention and reduction of visceral fat in rats [79]. In another study, Fernandes *et al.* has reported the efficacy of lowering cholesterol and triglyceride levels while improving HDL-cholesterol was seen in MC extract-fed diabetic rats [83]. In Nerurkar's laboratory experiments

using high-fat diet (HFD) fed mice demonstrated that MC juice lowered body weights, improved hepatic and plasma lipid as well as glucose metabolism [60]. It was further demonstrated that MC improved obesity-associated systemic and neuroinflammation in mice fed HFD [84]. Seeds of MC are also reported to have attenuation in body fat accumulation in HFD fed mice [85].

3.4 Clinical studies of MC

Compared with animal studies, there are limited numbers of clinical studies on the effects of MC. With the exception of the Tsai *et al.* study [86], all 23 clinical studies were conducted regarding the hypoglycemic effects of MC. The effect of hypoglycemia in diabetic people was first documented by Lakholia in 1956 [87]. Several clinical studies demonstrated that powder prepared from fresh MC juice and seeds significantly decreased blood glucose levels in T2D subjects [88-95]. Some studies used solvent-based extraction products of MC and showed significant hypoglycemic effects in type 1 diabetes (T1D) and T2D subjects [96-99]. On the other hand, Patel *et al.* [100] and several other studies demonstrated that MC fruit juice, powder, or tea did not show significant hypoglycemic effects [101-107]. Table 2 summarizes the overall descriptions of clinical studies. The majority of previous clinical studies on the effectiveness of MC was poorly designed and had short treatment durations (no longer than 6 months). Many studies lacked controls [86, 88-91, 97, 98, 100, 101], and only 5 out of 24 clinical studies were randomized double blinded clinical trials [93, 94, 104, 106, 108]. In addition to the poor

descriptions of subjects' baseline characteristics and study methodologies, little or no statistical analysis was conducted [88, 93, 98-100, 103, 109].

So far, there are no clinical studies investigating the effects of MC on obesity. One of the most recent studies by Tsai *et al.* focused on MetS and MC. Tsai *et al.* conducted open-labeled, clinical trials in MetS on adults. Participants in the study consumed about 5.0 g of dry powdered MC fruit and seed capsules for 3 months with a 3 month follow up period following the treatment. The results showed a significant reduction in the incidence of MetS, also a decrease in the waist circumference after 3 months of supplementation with MC powder without any major adverse events. In addition, effects of MC supplementation were only observed for the first month after discontinuation of the supplement period [86].

Table 2. 24 clinical studies of *Momordica charantia* (MC) (from the newest to the oldest by order)

Reference	Study design	Subjects	MC form	Treatment duration	Outcome measurement	Statistical significance/ Results
Tsai, *et al*., 2012 [86]	preliminary open-label un-controlled clinical trial	n=42 with three or more conditions of MetS criteria; n=38 completed	capsules from dried fresh wild MC fruits &seeds; 480mg/ capsule; 4.5-5.0g/day	3 months treatment + 3 months follow-up	- a decline in the % MetS incidence - 5 diagnostic criteria for MetS and insulin resistance indicators	Yes; significant decrease in the incidence of MetS and decrease in the waist circumference after 3 months of supplementation and 1st month, but not 2 or 3 months in follow up
Hasan & Khatoon, 2012 [96]	randomized controlled trial	n=50 with T2DM (n=26 treatment and n=24 control)	tablets made from fresh whole MC fruits; 1g/tablet; 6g/day	4 weeks	- fasting plasma glucose - post prandial plasma glucose - fructosamine	Yes; significant lower serum glucose levels, significant decrease on serum cholesterol and TG levels
Fuangchan, *et al.*, 2011 [94]	randomized double blind clinical trial	n=129 with newly diagnosis with T2DM (n=22 control, n=33 MC500 mg/day, n=32 MC1000 mg/day, n=31 MC2000 mg/day); n=120 completed	capsules from dried fresh fruit pulps; 500mg/ capsule	4 weeks	- mean change in fructosamine - mean change in fasting plasma glucose and 2-h post OGTT	Yes; significant decline in fructosamine at week 4 of the control and MC 2000mg/day group

Reference	Study design	Subjects	MC form	Treatment duration	Outcome measurement	Statistical significance/ Results
Lim, *et al.*, 2010 [93]	randomized double blind clinical trial	n=40 with T2DM (control, 60mg,, 80mg ,100mg /kg/day)	tablets from dried MC leaves obtained from local licensed pharmacy; 500mg/ tablet	single-treatment	- change in glucose and insulin level 4 hrs after the treatment	Yes; significant higher insulin levels and significant lower plasma glucose levels for the 100mg /kg/day
Fuangchan, *et al*., 2009 [92]	cohort study	n=82 with T2 DM, taken >1 oral hypo-glycemic drug; n=42 who had the same drug were evaluated	capsules made by dried powder of MC fruits ordered from a herbal company; 400mg/ capsule	77-315 days (median duration of 156 days)	- fasting plasma glucose	Yes; 45.2% had significantly reduced fasting plasma glucose levels for their target therapeutic level
Kasbia, *et al*., 2009 [106]	randomized double blind, crossover clinical trial	n=5 non-diabetic overweight men	freeze dried juice of MC; 50mg/kg/ body wt & 100mg/ kg/body wt	single-treatment for 3 weekly visits	- plasma glucose and insulin concentration - energy expenditure rates - appetite score	No
Rahman, *et al*., 2009 [107]	clinical trial	n=50 with T2DM	Juice from fresh unripe MC fruit	single treatment	- serum sialic acid concentration - total cholesterol, glucose, triglyceride concentration	No

Reference	Study design	Subjects	MC form	Treatment duration	Outcome measurement	Statistical significance/ Results
**Khadija & Aziz, 2009 [108]	randomized double blind clinical trial	withdrawn	not available	not available	-fructosamine -development of major adverse effects - GLP-1 [7-36] - fast blood glucose - HOMA-IR - insulin resistance	not available
Dans, *et al.*, 2007 [104]	randomized double blind clinical trial	n=40 with newly diagnosed or poorly controlled T2DM	capsules made from MC fruits and seeds obtained from a herbal company	3 months	- change in the HbA1C level - effect on fasting blood sugar, serum cholesterol, and weight	No
*Purificacion, *et al.*, 2007 [111]	not available	not available	not available	not available	not available	not available
Tongia, *et al.*, 2004 [99]	controlled trial	n=15 with T2DM (divided into three groups)	methanol extract powder of whole MC fruits (+ pulp and seeds)	1 week	- fasting and 2-hr post prandial blood glucose levels	Yes: significant hypoglycemic effect in methanol extract MC powder and half dose of metformin or glibenclamide or both in combination
John, *et al.,* 2003 [103]	randomized clinical trial	n=50 with T2DM (n=26 treatment and n=24 control)	tablets made from dried whole fresh MC fruits; 1g/tablet; 6 g/day	4 weeks	- fasting plasma glucose and post prandial plasma glucose -fructosamine	No

Reference	Study design	Subjects	MC form	Treatment duration	Outcome measurement	Statistical significance/ Results
Rosales & Ferrando, 2001 [102]	open label, crossover clinical trial	n=27 with T2DM (n=14 treatment & n=13 control); n=23 completed	tea prepared from MC fruits	24 weeks (12 weeks each trial)	- mean change in HbA1c, fast blood sugar, and SGOT	No
Ahmad, *et al*., 1999 [91]	case series	n=100 with moderate T2DM	fresh juice from MC fruits	single-treatment	- fasting glucose - 2-hr post OGTT	Yes; significant reduction of fasting and post-prandial serum glucose level
Srivastava, *et al*., 1993 [98]	case series	n=12 with DM	1) aqueous extract of MC fruits	3-7 weeks	1) HbA1c and post prandial blood glucose 2) post prandial blood glucose	1) Yes; significant in hypo-glycemic effect
			2) powder of dried fruits			2) No
Grover & Gupta, 1990 [90]	case series	n=14 with T2DM & n=6 with T1DM	seeds	single-treatment	- post prandial blood glucose	Yes; significant reduction in post prandial blood sugar
Welihinda, *et al.*, 1986 [89]	case series	n=18 with T2DM	juice from MC fruits without seeds	single-treatment	- 2-hr post OGTT	Yes
Akhtar, *et al.*, 1982 [101]	case series	n=8 with DM	juice from MC fruits	1 week	- fasting glucose level - glycosuria - 2-h post OGTT	No
Leatherdal, *et al*., 1981 [88]	case series	n=9 with T2DM	1) fresh juice from MC fruits; 50ml	1) single treatment	1) 2-hr post OGTT	1) Yes; reduced the plasma glucose

Reference	Study design	Subjects	MC form	Treatment duration	Outcome measurement	Statistical significance/ Results
					2) HbA1c	
				2) 8-11 weeks		2) No
			2) fried MC fruits; 230g			
Khama, *et al*., 1981 [97]	case series	n=8 with T2DM & 11 with T1DM	poly-peptide-p' isolated from whole MC fruits & seeds	single treatment	- blood glucose	Yes; hypo-glycemic effect of polypeptide-p
Baldwa, *et al.*, 1977 [96]	controlled trial	n=14 with DM(T1& T2) & n=5 healthy volunteers	purified protein extract from fruit and tissue cultures of MC, then homologous to animal insulin	single treatment	- blood glucose	Yes; significant decrease in average blood glucose throughout 12 hours
Patel, *et al.*, 1968 [100]	case series	n=15 with DM	fresh juice &dried powder of MC fruits	6-14 weeks	- 2-hr post OGTT	No
Lotlikar & Rao,1966 [110]	not available	not available	not available	not available	not available	not available
*Lakholia, 1956 [87]	not available	not available	fresh juice (up to 1oz/day)	not available	not available	Yes; significant disappearance of all the symptoms of DM, reduction in urinary sugar

* no online source available
* * study withdrawn

3.5 Adverse effects of MC

No large-scale studies have been undertaken to demonstrate long term safety of MC. The most commonly reported side-effects with consumption of MC fruits are gastrointestinal complaints including abdominal pain, bloating, and diarrhea [86, 104]. In some cases headaches and dizziness were also reported [94].

A few cases of serious side effects have been noted with consumption of MC leaves and seed extract but not fruit. Two hypoglycemic coma cases were reported in 3 and 4 years old children who ingested water extract of MC leaves and vines [112]. Also, MC may cause a potential risk of abortion when consumed by pregnant women [57]. Regardless of these few adverse events, Raman and Lau suggests that it is hardly realistic for a human to consume a life-threatening dose of MC, estimated in the range of 400 ml to 1,000 ml MC fruit juice based on animal experiments [112]. The optimum dosage of MC for human consumption has not been established because of the wide methods of preparations and the different varieties of MC [113]. Traditionally, Ayurveda recommends consumption of 30 to 60 g of fresh MC juice twice daily for diabetic subjects [60]. Overall, the adverse effects of MC fruits are minor, and most of the clinical studies seek to determine the anti-diabetic and anti-obesity potential of MC fruit.

Chapter 4: Hypothesis, Objectives

4.1 Hypothesis

Central hypothesis: Long- term consumption of MC fruits on a daily basis promotes weight loss and improves lipid profiles without major side effects in healthy overweight and obese individuals.

4.2 Objectives

Objective 1: determine if individuals will consume MC fruit powder for up to one month, regardless of taste preferences.

Working hypothesis: Snee *et al.*'s pilot study demonstrated willingness to consume MC was based on taste and health information of MC, and did not impact the Stages of Change (SOC) for consumption intentions in young, healthy adults [55]. We ***hypothesize*** that overweight and obese people with a desire to lose weight will consume MC everyday regardless of its bitter taste.

Objective 2: to investigate safety and efficacy of MC fruit powder on body weight, plasma glucose and lipid profiles.

Working hypothesis: Animal and cell culture studies [60, 63, 83] demonstrate that MC fruit juice improves hepatic and plasma lipid metabolism and reduces weight gain in high-fat diet fed mice.

One clinical study demonstrated that MC decreased in waist circumference and incidence of MetS in individuals with three or more conditions of MetS criteria [86]. We therefore ***hypothesize*** that MC will reduce body weights, and improve lipid profiles in healthy overweight and obese individuals without major adverse effects.

Chapter 5: Materials and Methods

5.1 Protocol Approval

The study was reviewed and approved by the University of Hawai‘i’s Committee on Human Studies (CHS). CHS # 16131.

5.2 Preparation of MC powder

Fresh, unripened Chinese bitter melons (*Momordica charantia* L.; MC) (Figure 1) were purchased from local markets in Chinatown, Honolulu, Hawai‘i. This specific variety of MC was selected based on the availability and popularity in Hawai‘i and our previous findings in cell culture and animal models [60, 63, 83]. A total of 40.9 kg of fresh MC fruit with seeds were washed and deseeded. The deseeded fresh MC was pureed using a household blender and stored at -20 °C until freeze-dried. MC was processed in a certified kitchen at the department of Human Nutrition, Food and Animal Sciences (HNFAS), University of Hawai‘i. Three separate batches of MC puree were freeze-dried for 48 hours, using a different lyophilizer from previously published protocol [60, 63]. It should be noted that the yield of dry MC powder from raw MC had different weights from each. All batches of MC powder were frozen at -20 °C until used for the feeding study.

Figure 1. Chinese variety of *Momordica charantia* fruits.

http://www.bikudo.com/product_search/details/109220/bitter_melon_extract.html.

5.3 Preparation of placebo powder

Iceberg lettuce was chosen as a placebo based on its similar color to MC fruit. Fresh iceberg lettuce was purchased from a local farmer's market in China town, Honolulu, Hawai'i. Iceberg lettuce was washed, leaves were separated and stalks removed. Iceberg lettuce was pureed, freeze dried similar to MC and stored at -20 °C.

5.4 Study subjects and design

5.4.1 Subject Recruitment

Subjects aged 18 to 70 years were recruited at the University of Hawai'i at Mānoa (UHM) campus by advertising via paper flyers (Appendix A) posted in the Agricultural Sciences Building, the Biomedical Sciences Building, Gilmore Hall, UHM Health Services, and on a bulletin board in the John A. Burns School of Medicine from July 2011 to July 2012. An initial screening interview was conducted over the phone or in person to determine the subjects' eligibility using the survey form designed by Dr. Nerurkar during grant submission (Appendix B). Eligibility and inclusion criteria were:

1) Calculated BMI above 25 kg/m^2

2) No health conditions of pre-diabetes and/or diabetes and hypoglycemia,

3) No abnormal lipids (TGs, low density LDL-cholesterol, serum CH, or low high density HDL-cholesterol)

4) No history of liver and/or kidney disease

5) No food allergies and not following a diet or restrictions and/or a specific diet plan

6) Not taking medications or over the counter supplements.

Subjects failing to meet the eligibility criteria were excluded. Exclusion criteria were any one of:

1) Calculated BMI below 25 kg/m^2

2) Presence of disease conditions including pre-DM and/or DM, dyslipidemia, liver and/or kidney diseases

3) Following a special diet or diet restrictions

4) Intake of prescribed medications

5) Women who were pregnant or lactating.

6) Children under 18 years old.

5.4.2 Study design

1) Feasibility study

Part A

Subjects were asked questions to determine willingness and intent to consume MC for one month during the initial paper survey (Appendix B). These questions were 1) Q.13 "Do you eat bitter

tasting foods and vegetables? If yes, what type and how often per week?" 2) Q.14 "Have you ever eaten bitter melon? If yes, did you like it?" 3) Q15. "This study involves eating bitter melon-containing dishes (1 cup) once every day for a total of one month. Do you have any concerns about being able to do this?", and 4) Q 16. "How many times would you be willing to eat bitter melon in 2 weeks if you knew it had potential health benefits?"

Part B

Compliance of daily consumption of MC was assessed in the clinical study by collecting unconsumed MC powders and subject's self-report.

2) Clinical study

The clinical study was designed as a four week, randomized, double-blind, placebo control trial with both placebo and treatment groups. After the completion of the initial survey, subjects who were eligible to participate in the clinical study were required to make four visits to the Agricultural Sciences Building, UHM. During the first visit the study procedures were orally described and provided in a written consent form. In addition to the written consent form, anthropometric measurements were obtained from the subjects. Anthropometric measurements included height, weight, waist and hip circumference, and body fat and lean body mass percentages estimated by an electronic scale (Omron HBF-500 Body Composition MONITOR with Scale). Before scheduling the next visit, subjects were referred to the Diagnostic Laboratory Services (DLSs) in Honolulu, Hawai‘i for an initial blood analysis. Then, subjects

were scheduled for a second visit and given either MC or placebo powders equivalent to 50.0 g of fresh MC or lettuce. Each dosage of dried MC powder was carefully measured using an electronic scale and placed into a 2 ounce plastic container. Subjects were asked to consume MC or lettuce powder once a day at dinner time with sugar-free lemonade supplied by the study. Blood analysis and anthropometric measurements were scheduled to be conducted at baseline (week 0), week two, and week four throughout the study period. Study subjects were asked to maintain the same diet and lifestyle. Subjects received a $ 20 gift card for each blood draw, and an additional $ 40 gift card at the completion of the study.

Blood chemistry

Blood samples were collected for the primary outcome measurements of the clinical study. Lipid analysis included TG, serum CH, LDL-cholesterol and HDL-cholesterol. Other blood measurements including fasting glucose, Apolipoprotein B, creatinine, asparatate amino transferase (AST), alanine amino transferase (ALT), alikaline phosphatase, total bilirubin, total protein, and albumin were also analyzed to measure dyslipidemia, liver and kidney function.

Dietary assessment

Initially, three 24-hour dietary records (Appendix C) were requested from the subjects during the study period. Dietary variables included total calories, protein, carbohydrates, dietary fiber,

cholesterol, total fats, and servings of FV intake. Dietary analysis of these 24 hour dietary records was performed with the Food Processor for Windows © version 8.4.0 (ESHA Research, Salem, Oregon). Dietary records were collected to determine the subject's "typical dietary habits" and to determine whether MC was associated with weight loss or results were confounded by other dietary factors such as FV amongst overweight and obese individuals.

Chapter 6: Results of the study

6.1. Subject Demographics

As observed in Table 3, a total of 25 subjects were recruited for the study (M=48%, F=44%, unknown 8%). The population demographics of total subjects is summarized in Table 3. Nearly one out of three participants were Chinese (32%), followed by Japanese (12%), and Filipino (12%). Other ethnicities included Caucasian, Japanese-Taiwanese, Hawaiian/part-Hawaiian, and Korean. Approximately one out of three participants was between the ages of 25-29, followed by 18-24, 40-49, 30-39, 50-59, and 60-70 years (Table 3).

Table 3. Subjects demographics of the whole study (n=25)

Characteristic	n	%
Gender*		
Male	12	48
Female	11	44
Unknown	2	8
Ethnicity**		
Caucasian (white)	2	8
Chinese	8	32
Japanese	3	12
Japanese/Taiwanese	1	4
Hawaiian/Part-Hawaiian	1	4
Filipino	3	12
Korean	1	4
Unknown	6	24
Age (yr)***		
18-24	3	12
25-29	8	32
30-39	1	4
40-49	3	12
50-59	1	4
60-70	2	8
Unknown	7	28

*gender provided by 23 participants
**ethnicity provided by 19 participants
***age provided by 18 participants

6.1.1 Recruitment of eligible candidates for the study

Of 25 subjects, 19 subjects completed all or the part of the initial survey and 6 subjects did not. The reasons for not completing the initial survey were:

1) No contact at the initial survey (n=5).

2) Lost interest participating in the study (n=1).

Among the 19 subjects who completed the initial survey and were further assessed for eligibility in the clinical study, 13 subjects were excluded based on the initial survey. The reasons for exclusion were:

1) Six subjects had a BMI of less than 25.

2) One obese subject had prediabetes, usage of medications, and was following a diet plan

3) One subject had health complications including high HbA1C, fasting plasma glucose (FPG), and LDL-cholesterol with BMI of less than 25.

4) One subject had a history of pre-diabetes and was following a Blood Type Diet, established by Dr. Peter J. D'Adamo based on blood type [114].

5) Four subjects did not provide their height and weight.

Table 4 summarizes the reasons for withdrawal.

Table 4. Reasons for exclusion from the clinical study

No initial survey (n=6)
1) No contact at the initial survey (n=5) 2) Lost interest during the initial survey (n=1)
Based on the result of the initial survey (n=13)
1) BMI less than 25 (n=6) 2) High HbA1c, FPG, LDL-cholesterol and BMI less than 25 (n=1) 3)History of prediabetes, usage of prescribed drugs, following a diet plan (n=1) 4) History of prediabetes, following blood type diet, and BMI less than 25 (n=1) 5) No data for height and weight (n=4)
Based on the result of initial blood draw (n=5)
1) High CH, LDL-cholesterol (n=3) 2) High CH, TGs, LDL-cholesterol (n=1) 3) High SGOT (AST) (n=1)

Six subjects met the inclusion criteria at the initial survey and initially enrolled in the clinical study, however 4 subjects were excluded after the initial blood analysis due to high CH and LDL-cholesterol (n=3) and high CH, LDL-cholesterol, TG, and low HDL-cholesterol (n=1). In addition, one subject was excluded due to higher SGOT (AST) regardless of normal lipid profiles. Therefore, only 1 subject (subject 6) participated in the trial. Table 5 and 6 show baseline characteristics of the 6 subjects. Figure 2 explains the overall flow of the subject recruitment and the feasibility and the clinical trial.

Table 5. Baseline characteristics of anthropometric measurements of subjects 1 to 6.

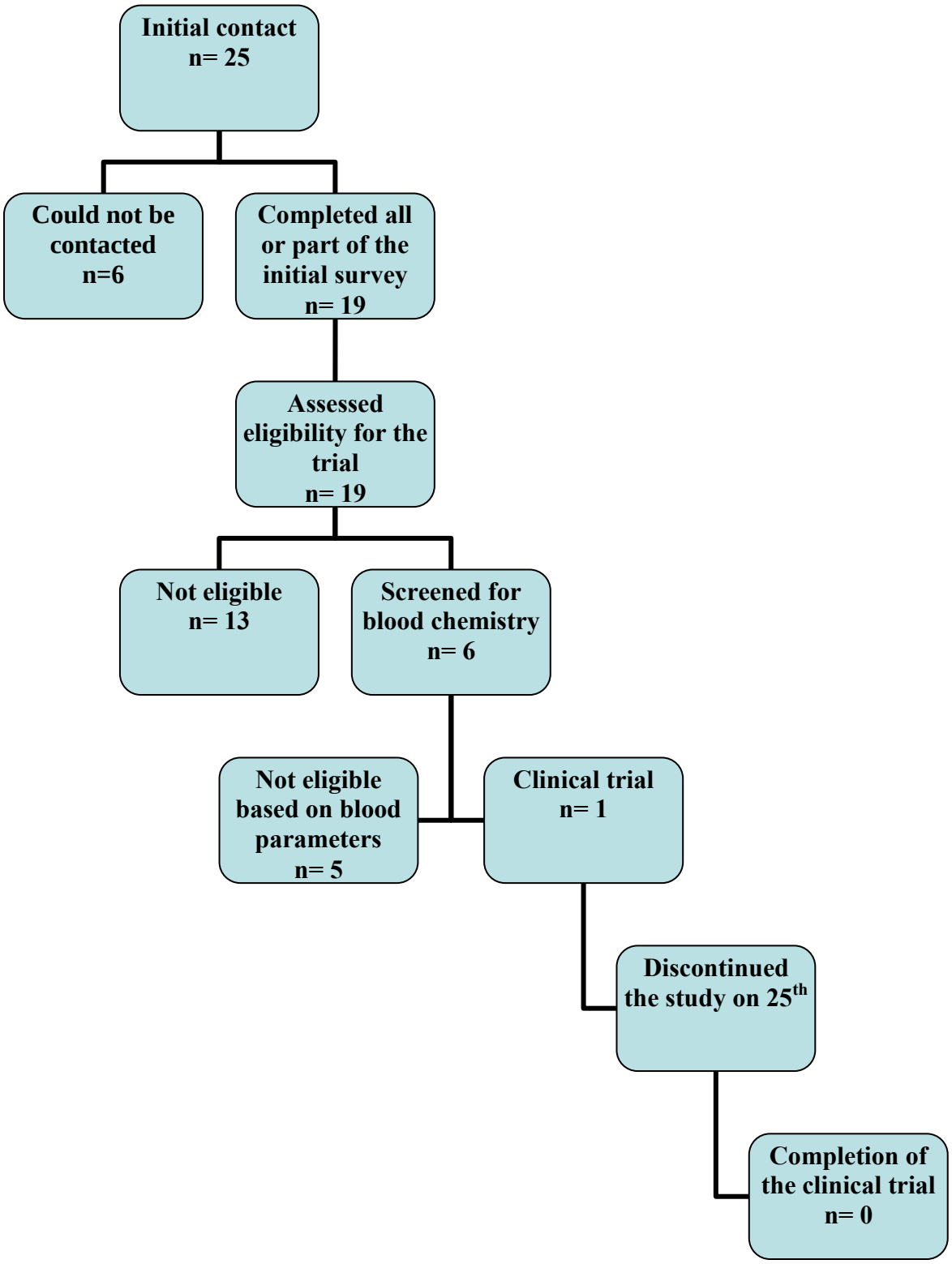
Variable	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6
Gender	M	M	M	F	M	M
Age	27	26	45	44	22	22
Body weight (kg)	94.5	95.0	80.0	75.0	101.0	103.0
Body mass Index (kg/m2)	29.0	31.9	28.7	31.1	34.3	30.7
Waist circumference (cm)	94.7	108.0	93.0	91.4	102.9	101.6
Hip circumference (cm)	97.8	101.6	99.1	99.1	100.8	101.6
Fat mass (%)	24.9	30.7	24.9	41.0	24.2	22.7
Lean body mass (%)	36.7	34.2	36	26.5	37.7	37.7

Table 6. Baseline characteristics of blood chemistry of subject 1 to 6.

Variable	Normal range	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6
Glucose (mg/dl)**	**70-99**	96	82	80	90	86	87
Cholesterol (mg/dl)**	**<200**	**205***	**266***	**215***	**200***	155	131
Triglycerides **(mg/dl)	**<150**	79	101	**212***	81	89	55
HDL ** (mg/dl)	**≥40**	41	54	**32***	62	43	49
LDL (mg/dl)**	**<100**	**148***	**192***	**141***	**122***	94	71
Creatinine *(mg/dl)**	**Male: 0.6-1.2 Female: 0.5-1.1**	1.0	0.9	1.0	0.8	0.9	1.1
Apo-lipoprotein B* (mg/dl)**	**Male: 50-125 Female: 45-120**	N/A	**148***	111	88	N/A	59
SGOT (AST)* (IU/L)**	**5-40**	24	21	16	20	**41***	28
SGPT (ALT)* (IU/L)**	**5-35**	23	26	17	18	33	30
Alkaline Phosphatase *(U/L)**	**42-128**	84	45	60	52	72	46
Bilirubin, total* (mg/dl)**	**0.1-1.0**	1.0	0.8	0.5	0.2	1.0	**1.4***
Bilirubin, direct* (mg/dl)**	**0.1-0.3**	0.2	0.2	0.1	0.1	0.2	**0.4***
Bilirubin, Indirect* (mg/dl)**	**0.2-0.8**	0.8	0.6	0.4	0.1	0.8	**1.0***
Total protein* (gm/dl)**	**6.4-8.3**	6.9	7.2	7.6	7.2	7.3	7.0
Albumin* (gm/dl)**	**3.5-5.0**	4.2	4.6	4.4	4.1	4.4	4.6

*higher than normal value
** reference range from the Diagnostic Laboratory Services, Honolulu, HI
*** reference range from Pagana, K.D., & Pagana, T. J., 1997. [115]

Figure 2. Subjects flow through the recruitment to the feasibility and the clinical trial



6.2 Feasibility study

Part A

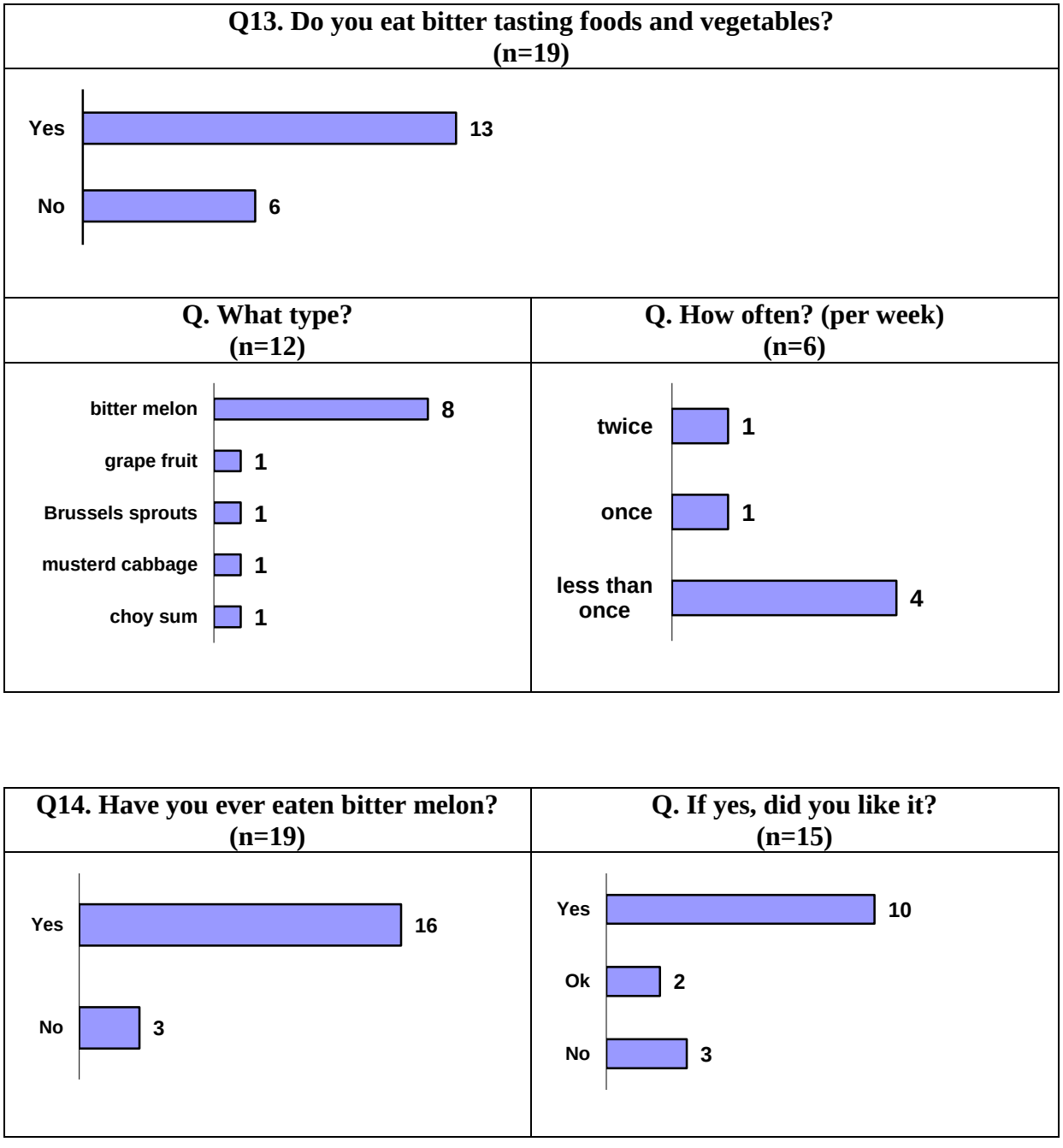
Of 19 subjects who participated in the feasibility study, 68 % (n=13) indicated that they eat bitter tasting foods and vegetables, while 32 % (n=6) do not eat bitter tasting foods and vegetables. Of the 13 subjects who eat bitter tasting foods and vegetables, 67 % (n=8) indicated they eat MC. Grape fruit, mustard cabbage, Brussel sprouts, and choy sum were also mentioned as types of bitter tasting foods and vegetables that are consumed. However, 30 % (n=4) of the 13 subjects who eat bitter tasting foods and vegetables indicated that they eat bitter tasting foods and vegetables less than once a week, followed by once a week (n=1), and twice a week (n=1), respectively. Meanwhile, 54% (n=7) of those 13 subjects did not indicate how often they eat bitter tasting foods and vegetables.

Of the19 subjects, 84 % (n=16) indicated they have eaten MC before, while 16 % (n=3) had never eaten MC. In addition, 53 % (n=10) indicated that they liked MC and 10 % (n=2) accepted (but did not indicate liked) MC, while 16 % (n=3) did not like MC. Meanwhile, 21 % (n=4) did not indicate whether they liked MC or not.

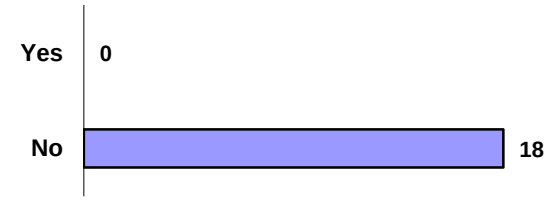
All 18 subjects, except one subject who did not complete the questions 15 and 16, indicated they had no concerns with participating in a clinical study involving consumption of MC once a day for one month. Of those 18 subjects, half of the population (n=9) indicated they were willing to eat MC 2 or 3 times over a 2 week period knowing the health benefits, 39 % (n=7) showed a willingness to eat MC more than 4 times in 2 weeks. Two subjects (11%) indicated that they

would eat MC once in 2 weeks regardless of knowing any potential health benefits of MC. Figure 3 illustrates the statistics for the answers of the feasibility questions.

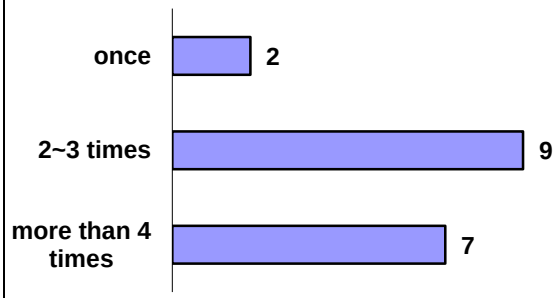
Figure 3. Summary of statistics of answers for feasibility questions.



Q15. This study involves eating bitter melon-containing dishes (1 cup) once every day for a total of one month. Do you have any concerns about being able to do this? (n=18)



Q16. How many times would you be willing to eat biter melon in 2 weeks if you knew it had potential health benefits? (n=18)



Part B

Of 25 subjects, only one subject participated in the feasibility study. Self reporting from the subject indicated daily consumption of MC was met on most of the days during the trial with the exception of missing MC consumption for 2 days. The subject also reported consuming the MC powder mixed with diet sprite, not sugar-free lemonade which was provided by the study.

6.3 Clinical study

6.3.1 A case report

A 22 year old Chinese male who completed the initial survey and underwent the initial blood draw met all the inclusion criteria. His baseline weight was 103 kg with a BMI of 30.7 kg/m^2. His waist and hip circumferences, and body fat percentage were 101.6 cm, 101.6 cm, and 22.7 %, respectively. In addition, his blood analysis and lipid profiles were within normal limits. His serum cholesterol was 131 mg/dl (normal < 200 mg/dl), his serum TGs were 55 mg/dl (normal <150mg/dl), his LDL-cholesterol was 71 mg/dl (normal < 100 mg/dl), and his HDL-cholesterol was 49 mg/dl (normal ≥ 40 mg/dl). His FPG was 87 mg/dl which was within a normal range (70-99 mg/dl, Table 7).

The study participant received a daily dosage of 3.0 g of dried MC powder (equivalent to 50 g of fresh MC fruit) and was requested to consume the powder with sugar-free lemonade at dinner

time. By 3 weeks, his weight reduced by 1.5 kg, and his waist and hip circumferences along with body fat percentage were reduced (Table 7). In addition, his serum TGs and LDL-cholesterol decreased by 31 % and 6 %, respectively, while HDL-cholesterol increased by 14 % after 3 weeks of MC treatment. Also, FPG slightly increased by 6 %, but remained within a normal range. No change in serum CH was registered. Even though no serious adverse events occurred during the trial, a slightly higher SGPT (ALT) value was observed at 3 weeks. Thus the subject was reported to discontinue the trial on the 25th day since the cause of elevated ALT was unclear. Reports of medication and alcohol intake were not reported by the subject during the trial. However, the subject reported improvement of bowel movements and increased appetite during the trial.

Table 7 shows baseline anthropometric measurements and blood analysis, and the outcome measurements after 3 weeks of MC consumption by the subject.

Table 7. Baseline and Week 3 outcome measurements of the subject's anthropometric measurements, lipid profiles, and fasting plasma glucose levels.

	Baseline data	**21days after the MC intake**
Anthropometric measurements		
Weight (kg)	103.0	101.5 (1 % ↓)
Height (cm)	183	183 (0 %)
BMI (kg/m^2)	30.7	30.3 (1 % ↓)
Waist circumference (cm)	101.6	97.2 (4 % ↓)
Hip circumference (cm)	101.6	100.3 (1 % ↓)
Plasma chemistry		
Cholesterol (mg/dl)	131	131 (0 %)
Triglycerides (mg/dl)	55	38 (31 % ↓)
LDL-cholesterol (mg/dl)	71	67 (6 % ↓)
HDL-cholesterol (mg/dl)	49	56 (14 % ↑)
Fasting plasma glucose (mg/dl)	87	92 (6 % ↑)

6.3.2 Dietary analysis

A total of three 24 hour food records were obtained from the subject at week 0, week 2 and week 3. The subject's dietary habits did not vary substantially during the trial based on his food records. The subject had a relatively low consumption of total FV throughout the trial, consuming about 1.3 servings of fruit and 1.2 servings of vegetables per day at weeks 0, and 2.7 servings of fruit and 1.1 servings of vegetables per day at week 2. No serving of FV at week 3 was reported (Table 8). Accurate measurements of the subject's total kcals, protein, carbohydrates, dietary fiber, cholesterol, and fats were not obtained from the 24 hour food records due to insufficient or vague information about the food items consumed. However, estimate intake of protein, carbohydrates, and fat intake were calculated using the Food Processor and information from fast-food chain and casual dining restaurants (Table 8).

Table 8. Food lists and FV servings, estimated protein, carbohydrates, and fat intake which the subject consumed at week 0, week 2, and week 3.

Variable	Week 0	Week 2	Week 3
Breakfast	cooked rice (1 cup) pork sausage (3 strips) milk (1 cup) orange juice (1 cup)*	cereal (3 cups) milk (1 cup) doughnut (4) orange juice (2 cups)*	hot oatmeal (1 cup) cereal (4 cups) milk (1 cup)
Lunch	Zippy zip pack ** (from Zippy's) (1 serving)	Subway meatball mariana sandwich (12 inches long) potato chips * (1 small bag) iced tea (large)	ham & cheese sandwich (3) soda (large)
Dinner	Wendy's triple burger*** (1 serving) medium french fries* soda (20 fluid oz)	steak (32 oz) pasta (1 plate) iced tea (20 fluid oz)	Zippy zip pack** (3 servings) soda (large)
Fruit (serving)	1.3	2.7	0.0
Vegetable (serving)	1.2	1.1	0.0
Protein (estimate)	151 g	352 g	235 g
Carbohydrates (estimate)	392 g	573 g	862 g
Fat (estimate)	165 g	108 g	179 g

* indicates major source of fruits and/or vegetables
* * Ingredients; chicken, fish, spam, teri, white rice, furikake, takuwan
* ** Ingredients; 3/4 lbs ground beef, cheese, mayo, ketchup, pickles, red onion, tomato 1-2 slices, lettuce, toasted bun

Chapter 7: Discussion

7.1 Reasons for difficulties in recruiting and retaining subjects

The clinical study was originally designed as a 4-week, randomized, double-blind, placebo controlled trial and the original goal was to recruit 50 subjects with 25 control individuals and 25 treatment individuals. However, the present study could only recruit a total of 25 subjects, with only one subject qualifying for the feasibility and efficacy and safety of daily MC consumption. The most common reason for exclusion, observed in 8 subjects, was a BMI less than 25 kg/m^2. This could indicate a high intention to lose weight among the subjects regardless of their current weight. Several studies have demonstrated that a desire to lose weight is found in persons having normal BMI [116-119]. Chang *et al.* indicated that 38.3 % of women with normal weight categorized themselves as overweight [119].

According to NHANES data from 2009 to 2010, nearly 70 % of adults in the U.S. were either overweight or obese [3]. In the present study, 47 % of the subjects were overweight or obese. In fact, Hawaii is the second lowest obesity prevalence state [11], and this outcome may reflect population demographics in Hawai‘i. Also, subjects were recruited via paper flyer on specific locations at the UHM. Sampling bias likely existed by posting a flyer in the locations where there were mostly medical and nutrition students and faculty who may be more conscious about health and nutrition than the general population. Also, the flyer did not provide concrete details about inclusion criteria. For instance, the term “generally healthy” was intended to mean disease-free. The wording of the flyer could be another reason more non-overweight/obese

subjects were recruited than overweight/obese individuals. These biases could have been reduced by selecting different locations for recruitment and providing more specific information about the study details.

The second major reason for excluding subjects was abnormal lipid profiles and a history of pre-diabetes. Four obese subjects reported abnormal lipid profiles and 2 subjects (one obese and the other one non-obese) had prediabetes. Excluding the subjects with abnormal lipid profiles, left only 2 obese individuals with optimal lipid profiles. These results demonstrate that obesity is highly correlated with dyslipidemia and hyperlipidemia. Nguyen *et al.* demonstrates that individuals with class 3 obesity were more than twice likely to have dyslipidemia compared with normal weight individuals [4]. The present study further excluded subjects who met any of the following criteria: taking prescribed drugs, following a special diet plan, or having a moderately high liver enzyme based on the exclusion criteria. Lastly, pregnant or lactating women were not included in the clinical study to avoid the risk of abortion reported in traditional medicinal usage of MC [57], and children were not included due to safety concerns [112].

7.2. Feasibility Study

Our results indicate that nearly 70 % (n=13) of the subjects ate bitter tasting foods and vegetables including MC occasionally, and approximately 85 % (n=16) of the subjects had eaten MC before. This finding exceeds the results from our previous palatability study of MC which

demonstrated 69 % (n=34) of participants had consumed MC before [55]. In addition, the present study demonstrates that approximately 65 % (n=12) of the subjects liked or accepted MC regardless of its bitter taste. This result indicates that MC is widely consumed and found acceptable among this sample population. However, this could be biased by people who knew what they were going to eat in the study. MC is primarily eaten in a variety of dishes in Asian countries, and despite its unpopularity as a vegetable in the Western world, Hawai‘i is one of a few places in the U.S. where MC is widely cultivated and consumed. The present study had almost 90 % of the subjects with Asian backgrounds, including Chinese, Japanese, Filipino, Korean, and Japanese/Taiwanese. Thus, it was not surprising to observe a high consumption of MC in such a population. However, the demographics of the subjects in the present study did not reflect the ethnic distribution of Hawai‘i. According to 2011 U.S. Census Bureau data, Asian population comprises 38.5 % of the whole population in Hawai‘i, followed by 26.0 % White, 22.9 % non-Hispanic White, 22.9 % multiple ethnicities, 10.1 % Native Hawaiian and other Pacific Island, 9.2 % Hispanic, 2.0 % Black, and 0.4 % American Indian and Alaska Native [120]. Therefore, the popularity of MC consumption in the present study might be due to the large representation of Asian ethnicity among the subjects.

Of the 18 subjects who responded with a willingness to consume MC for one month, all showed their willingness to participate in the MC feeding study. This result demonstrates that people’s intent to consume MC on a regular basis is realistic, and illustrates that MC can be potentially consumed on a long term basis as a potential obesity treatment. On the other hand, information about the health benefits of MC only had a small effect on consumption intentions. Thirty nine % subjects showed a willingness to consume MC for 4 or more times in 2 weeks after

information at health, while half of the subjects would consume MC only 2-3 times in 2 weeks and 11 % of subjects preferred to consume MC once in a 2 week period. This could possibly be a result of the subject's age since 44 % of the present study was under the age of 29. It is possible that younger individuals are less interested in the health benefits of functional foods such as MC. Interest and usage of CAM is highest in middle age adults between 50-59 years (44 %), while only 36.8 % for younger adults age 18-29 years [47]. This result was also similar to Snee *et al.*'s pilot study which demonstrated health information had no significant effect on intention to consume MC in young healthy adults [55]. Although 61 % of subjects in the present study had lesser intentions to consume MC, 39 % of subjects showed higher motivation to eat MC. This difference could be a consequence of nutritional knowledge. Ares *et al.* have demonstrated that both greater nutrition knowledge and health awareness are associated with individuals having high levels of interest and willingness to try functional foods [121]. In addition, Crites *et al.* found significant positive health evaluations and attitudes in subjects with knowledge of nutrition [122]. These findings suggest that the degree of nutritional knowledge influences levels of interest in health information. The present study recruited subjects who frequent nutrition and medical buildings in the University. Thus, it is likely that subjects with higher levels of nutritional knowledge and health awareness were recruited, and this could be reflected by subjects who had higher intentions to eat MC. Also, the flyer indicated the study involved MC, so it may have attracted people who ate MC.

Feasibility of MC consumption on a daily basis was demonstrated by one subject, Subject 6. Consumption of MC on a consistent basis by Subject 6 suggests the form of oral treatment could be realistic for daily MC consumption. In addition, the bitterness of MC did not discourage

consumption of MC. For example, the subject preferred mixing MC with diet sprite suggesting that commonly consumed drinks may increase feasibility of MC consumption. Subject 6 could not be contacted to collect the unconsumed MC treatment. Further exploration of the feasibility of daily MC consumption with a large sample population is needed for increased certainty.

7.3. Clinical Study

Many previous clinical studies of MC observed a hypoglycemic effect of MC [88-99]. These studies were focused on diabetic individuals only. Among all 24 clinical studies of MC, only one study was conducted with non-diabetic overweight men [106], and one other study recruited individuals with MetS [86]. Neither of these two studies measured lipid profiles nor body weights as their outcome measurement.

In Tsai *et al.*'s open-label, uncontrolled trial on MetS, the study found a significant reduction of waist circumference in Taiwanese individuals with daily consumption of 4.8 g dried MC fruit for 3 months [86]. MetS has a high correlation with obesity, and the result from Tsai *et al.*'s study suggests that daily intake of dried MC fruit for a long period of time is beneficial for reducing a risk factor of obesity prevalence. In the present study, Subject 6 who underwent MC treatment for 25 days had a reduction of weight and waist circumference, a decrease in TGs and LDL-cholesterol and an increase of HDL-cholesterol over 3 weeks of daily consumption of 3.0 g MC powder without apparent modification of diet and lifestyle changes. The result agreed with

the clinical study of Tsai *et al.* that MC might have a potential benefit for reducing abdominal obesity.

Kasbia *et al.* demonstrated that there was no effect on glucose and insulin levels by single MC treatment on non-diabetic overweight men [106]. Our study also observed daily consumption of MC powder had no effect on FPG on a healthy obese individual. These results suggest that moderate consumption of MC will not cause hypoglycemia, in non-diabetic individuals.

The present study observed elevation of SGPT (ALT) after 3 weeks of MC consumption beyond the normal range in subject 6 despite no serious adverse events reported from the subject during the trial. No history of clinical studies reported adverse hepatic events, including the recent MC clinical study that measured MetS [86]. SGPT (ALT) and SGOT (AST) are used to screen for hepatic diseases, and elevated ALT, often is used as an indicator of liver damage such as fatty liver disease. Prevalence of overweight and obesity, DM, hypertension, and consumption of alcohol also affect ALT levels. In addition, certain ethnic groups (for example, Filipinos have a higher prevalence of ALT elevation than other ethnic groups), gender (males are nearly twice more likely to have ALT elevation than females), and age and marriage status are also possible factors for elevating ALT [123]. Due to a limited number of participants in the present study and previous clinical trials on obesity-related topics, the efficacy and safety of MC for obesity treatment remains inconclusive.

Multiple 24 hour dietary records of Subject 6 demonstrated that his dietary habits remained relatively consistant thoughout the trial. This consistency indicates that the effect of weight loss

and reduction of waist circumeferences, a decrease in TG and LDL-cholesterol and an increase of HDL-cholesterol over 3 weeks were less likely to be influenced by dietary factors. In addition, the subject's low consumption of FV thoughout the trial indicates that those anthropometrics and lipid profile changes are less likely to be associated with intake of FV. Dyslipidemia, which is characterized by increased serum CH, LDL-cholesterol, and TGs, and decreased HDL-cholesterol [124], is one of the most common conditions observed in overweight and obese individuals. The dietary patterns of excess fats and high energy dense foods and beverages also contribute to the development of obesity [125]. On the other hand, a higher dietary intake of FV may aid weight management. Yet, FV consumption remains low among the U.S. adults even when the health benefits of FV are known. Only one in three (32.4 %) U.S. adults consume fruit at least twice daily and a quarter (26.3 %) of U.S. adults consume vegetables at least 3 times daily [126]. The USDA recommends daily consumption of at least 2 cups of fruit and at least 3 cups of vegetables for men aged 19 to 50 years old. For women in the 19 to 50 years old age range the daily recommended consumption of vegetables is 2 1/2 cups. The USDA recommends at least 2 cups of fruit per day for women aged 19-30 years old and 1 1/2 cups per day for women 31-50 years old [127].

Ledikwe *et al.* demonstrated that individuals who consumed a high fat diet and less than 5 servings of FV had the highest prevalence of obesity whereas individuals who had a high consumption of FV had lesser prevalence of obesity. The study further indicated that individuals who consumed more than 9 servings of FV had a low saturated fat intake and the lowest dietary energy density values [128].

Interestingly, Subject 6 who failed to meet the recommended intake of FV had no indication of hyperlipidemia. His lipid profiles at the initial blood draw indicated comparatively low levels of serum CH which is classified as hypocholesterolemia by American Heart Association [129]. The present study did not exclude Subject 6 from the clinical trial since the exclusion criteria did not indicate lower limit of serum CH. It has been documented that up to 30 % of obese individuals are 'metabolically healthy' which is characterized by absence of any metabolic disorders, and they seem to be protected from development of obesity-related morbidity [130]. The mechanisms of these protective effects against the development of metabolic disorders in healthy obese individuals still remain unknown. However, factors include genetics, physical activity, type and location of adipose tissues, age and gender. Overall, it is possible to interpret that the subject in the present study is a healthy obese individual.

Chapter 8: Conclusion

The findings of this study indicate that long-term MC consumption may be practical as an anti-obesity treatment. Previous exposure to MC and wide acceptance of MC to a majority of the subjects indicated a high level of intention to consume MC regardless of its bitterness. In addition, knowledge that MC may have health benefits slightly increased willingness to consume MC. Feasibility of MC consumption on a daily basis is possibly achievable with additional supplementation such as mixing MC with sweetened beverages which masks the bitterness of MC. The efficacy and safety of daily consumption of MC as an obesity treatment remain unknown. However, the fact that one subject showed improvement of lipid profiles and effects on weight loss and reduction of waist circumference in 3 weeks without any apparent dietary modifications and low consumption of FV suggests MC has potential benefits as an anti-obesity treatment. The effect of MC on liver enzymes may need further study.

Acceptance and willingness to consume MC is initially important and needs to be studied in a long-term feeding clinical trial. This study suggests that a large sample of randomized clinical trial design with overweight/obese individuals is needed to demonstrate efficacy and safety of MC. Further investigations of efficacy and safety of MC on obesity could indicate a possible alternative treatment to combat obesity, one of the most serious health problems that lead to a higher risk of mortality and morbidity in the U.S. and world wide. Also, as people have become more aware of CAM approaches to manage their health, a functional food such as MC has received attention for its potential efficacy in obesity treatment.

(Appendix A)

Food for thought:

Can adding bitter melon to your diet decrease your risk for diabetes and being overweight?

Researchers at the University of Hawaii are seeking volunteers to participate in a study to explore this answer.

You can participate if you are:	Benefits include:
• between 18 and 70 • generally healthy • not taking any prescription medications	• Free Diabetes screening • Free Cholesterol screening • Free BMI measurements

You will receive gift cards for your time.
There may be up to 4 study visits.

To schedule an appointment please call:
956-9195

956-9195	956-9195	956-9195	956-9195	956-9195	956-9195	956-9195	956-9195	956-9195	956-9195	956-9195	956-9195	956-9195

(Appendix B)

Completion of the clinical trial
n= 0

Bitter Melon Feeding Study

Date: ___/___/___(mm/dd/yr) **I.D. # __ __ __ __**

Name: _____ **DOB**: _____ (mm/dd/yr)

Gender:
□ Male
□ Female

Which ethnic or racial group below do you <u>most</u> identify with? Check only <u>one</u>.

Asian
□ Cambodian
□ Chinese
□ Filipino
□ Indian (from India)
□ Japanese
□ Korean
□ Thai
□ Vietnamese
□ Other (Write in) _____

Black
□ African American
□ Other (Write in) _____

Hispanic
□ Cuban
□ Mexican
□ Puerto Rican
□ Other (Write in) _____

Native American
□ American Indian
□ Alaskan Native

Native Hawaiian or Other Pacific Islander
□ Guamanian/Chamorro
□ Hawaiian/Part-Hawaiian
□ Samoan
□ Tongan
□ Other (Write in) _____

White
□ Portuguese
□ Other (Write in) _____

I.D. # __ __ __ __

1. Do you have a family history of diabetes, hyperlipidemia, high blood pressure or obesity?

Yes___; No___

2. Have you been diagnosed with any of the following health conditions:

A. Diabetes

Yes__; No___

If Yes, do you take any medications? Yes ___; No ___

What type of medications and for how long? _____

B. Hypoglycemia (low blood sugar with fainting, shaking, over hungry)

Yes___; No ___

If Yes, do you take any medications? Yes___; No ___

What type of medications and for how long? _____

C. Chronic arthritis or any inflammatory diseases

Yes ___; No ___

If Yes, do you take any medications? Yes ___; No ___

What type of medications and for how long? _____

D. High or low blood pressure

Yes__; No___

If Yes, do you take any medications? Yes ___; No ___

What type of medications and for how long? _____

E. Abnormal (high or low) lipids (high triglycerides, LDL-cholesterol, total cholesterol, VLDL-cholesterol or low HDL-cholesterol)

Yes__; No___

If Yes, do you take any medications? Yes ___; No ___

What type of medications and for how long? _____

I.D. # __ __ __ __

4. Do you drink alcohol?

Yes ___; What kind:_____ and How often: _____ No ___

5. Have you undertaken any blood tests recently?

Yes ___; What kind: _____; When and where: _____

No ___

6. Are you on any type of dietary restrictions or following any special dietary plan for health conditions or religious reasons?

Yes ___; Type of diet: _____

No ___

For how long have you been following this diet plan? ___ month/years

Was this diet recommended by a doctor or other health care professional?

Yes ___ No ___

7. Do you use any type of alternative or traditional medicine?

Yes ___; Type of medications: _____

No ___

8. Do you use over the counter supplements?

Yes ___; Type of supplements: _____

No ___

9. Are you pregnant or plan to be pregnant in next six months? (Only for female subjects)

Yes ___ No ___

10. Have you been hospitalized?

Yes ___ No ___

If yes, provide brief summary _____

I.D. # __ __ __ __

11. Have you had surgery?

Yes ___ No ___

If yes, provide brief summary _____

12. Do you have any food allergies?

Yes ___ No ___

If yes, what food(s)? _____

13. Do you eat bitter tasting foods and vegetables?

Yes ___; What type: _____;

How often: _____ (per week)

No ___

14. Have you ever eaten bitter melon?

Yes ___; Did you like it: _____

No ___

15. This study involves eating bitter melon-containing dishes (1 cup) once every day for a total of one month. Do you have any concerns about being able to do this?

Yes ___ No ___

If yes, what are your concerns? _____

_____

16. How many times would you be willing to eat bitter melon in 2 weeks if you knew it had potential health benefit?

Once ____ 2-3 times ____ 4 or more times ____

17. Will you be able to come and pick up food once a week and keep it frozen at home? (We will provide you coolers and blue ice packs which you will return at the end of the study)

Yes ___ No ___

Lab Requirements

18. You will be asked to donate 30 to 35 ml of blood (5 small tubes) after an overnight fast three times during the study. You will receive $20 on the day of collection for a total of $60. Do you foresee any problem in participating in this part of the study?

Yes ___ No ___

If yes, what? _____

_____

19. You will be asked to keep a 3 day record of all the foods and medicine you consume as well as physical activity. Do you foresee any problem in participating this part of the study?

Yes ___ No ___

If yes, what? _____

_____

Other Concerns

20. Do you foresee any difficulty in participating in this study?

Yes _____;

What: _____

_____

_____.

No _____

(Appendix C)

University of Hawai‘i at Mānoa –The Bitter Melon Human Study
24 hours Dietary Record

Name_____ **Date_____/_____/_____**

Gender_____ **Age_____**

Approximate Weight_____ **Height_____**

Direction: Please be as specific as possible, measurable units. Include all beverages, condiments, and portion sizes in measurable units (ex: cups, grams, oz, Tbsp, tsp, small, medium, etc).

Example: Hamburger and soda

Time	**Food item and method of preparation**	**Eaten amount**	**Where**
11am	Grounded beef patty, pan fry	5oz	Burger company
11am	Whole-wheat ban, toasted	3oz	Burger company
11am	tomato	2 slices	Burger company
11am	Swiss cheese	1oz	Burger company
11am	alfalfa	30g	Burger company
11am	Diet coke	12oz	Burger company

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