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No Effect of Momordica Charantia Linn on Glycemic Regulation, Energy Expenditure and Appetite in Healthy Overweight Men: A Pilot Study

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NO EFFECT OF MOMORDICA CHARANTIA LINN. ON GLYCEMIC REGULATION, ENERGY EXPENDITURE AND APPETITE IN HEALTHY OVERWEIGHT MEN: A PILOT STUDY

Thesis

Submitted to the Faculty of Graduate and Postdoctoral Studies in partial fulfillment of the requirement for the degree of

Masters of Science in Human Kinetics

University of Ottawa

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Introduction

"Let food be thy medicine, and let medicine be thy food." -Hippocrates

Since the existence of man, challenges such as diseases have often claimed lives or decreased quality of life. Food was scarce and man typically ate with a survival type diet filled with nuts, and protein. Mortality was high, but survival was the key concern as the elements such as ice and snow could easily contribute to disease and death. Slowly, organized civilizations such as the early Sumerians found plants which may aid certain diseases. Those who administered these were deemed medicinal healers and were sought for their wise understanding of the distinct relationship between nature and healing.

This is a far contrast from the pharmacological culture which has developed globally. In today's age society looks for quick ways to fix issues that often have been the result of poor dietary practices and hygiene. Companies design drugs which can "cure" the body, without realizing the true balance of nature. Some of these drugs provide more side effects that can even worsen the quality of life of many who they are prescribed too. The irony is that many of these drugs have been derived from plants. For instance the world's greatest prescribed diabetic drug "metformin" is derived from the French lilac (*Galega officinalis*).

This being said, some fruits offer similar medicinal qualities, such as the reduction of high blood sugar, normalizing of lipo-proteins, and the protection of specific tissues within the body. While some societies currently depend on pharmacotherapy, others may not have the monetary capacity to do so. Thus dietary intervention for many diseases is considered as medicine, which is a concept that has been lost since the initial inception of modern allopathic medicine by the father of medicine Hippocrates. The content of this thesis first reviews the effects of a plant,

Momordica Charantia Linn. (MC), in relation to the treatment of type II diabetes. Secondly, the results from a pilot study sought to examine the acute effect of MC on post-prandial glucose levels, energy metabolism and appetite in normal body weight individuals are presented.

Abstract

Phytomedicines are currently being researched for the treatment of various pathologies, including those involving carbohydrate and lipid metabolism. *Momordica Charantia Linn* Cucurbitaceae (MC), otherwise known as ``Bitter Melon``, Nigauri (Japan), Sorrow See (Caribbean) and Karela (India), has been used to treat glycemic impairment in relation to diabetes for centuries. The objective of this study was to determine the acute effect of MC on post-prandial glucose levels, energy expenditure/ fuel mixture and appetite in normal body weight individuals. Five healthy men (34 ± 14 SD y; 27 ± 0.34 SD kg/m^2 BMI) were supplemented on three randomized conditions where 1) no MC (Ctl), 2) 50 mg/kg g of glucose (MC50) or 3) 100 mg/kg of freeze dried MC (MC100) were given orally prior to a 75 g oral glucose tolerance test (OGTT). Plasma glucose and insulin levels were measured before and during the OGTT. Energy expenditure as well as carbohydrate and lipid oxidation rates were measured by indirect calorimetry. Finally, visual analogue scales were used to rate appetite. Plasma glucose and insulin levels significantly increased during the OGTT ($P < 0.05$) but no significant difference was observed between experimental conditions. Energy expenditure did not change over time, regardless of the experimental conditions. During the OGTT, a shift from lipid to carbohydrate oxidation was observed, regardless of the experimental conditions. Finally, no treatment effect was found for appetite scores throughout any of the experimental sessions. These results suggest that from an acute standpoint MC does not affect plasma glucose and insulin levels, energy expenditure, substrate mixture and appetite scores following an oral glucose load in healthy men.
Keywords: Phytomedicine, Obesity, *Momordica Charantia L*inn, Energy Expenditure, Appetite, Humans, Visual Analogue Scale, Hunger.

In recent years, obesity has become a growing worldwide problem contributing to a wide array of pathologies including diabetes. *Momordica Charantia Linn.* (MC) is a plant used by

traditional tribal healers, Ayurvedic and Chinese traditional complementary medicine (TCM) practitioners. MC has been found to have many medicinal properties in relation to the treatment of type II diabetes and insulin resistance. In recent years specific phytoactive components have been revealed which may link this fruit to protective and normalizing attributes for type II diabetes. While MC has the potential to be used as a treatment for insulin resistance, literature reveals that further clinical assessments are required to fully evaluate its acute efficacy and long term impact. Furthermore, future research within this area must move into a clinical setting, whereby specified standardized extracts and genotypic characteristics of MC must be taken into consideration when reporting data. Relevant information on MC in the context of obesity and insulin resistance along will be discussed in this review.

Key Words: *Momordica Charantia L.*, Diabetes, Obesity, Phytomedicines,

Definitions:

Saponins: The glycoside portion of steroids, steroid alkaloids or triterpines found in plants, linked to medicinal qualities associated with cholestoral reduction.

Review of Literature

A growing interest in alternative medicine has led to continuing research on phytomedicines. Current allopathic medications may have many unwanted side effects associated with them, and can cause medical complications of their own. Clinicians and patients are now looking for alternatives to use, for treatment of pathological conditions they may have. A particular pathology of global significance is obesity. The complications of obesity are

monumental, in that it can set the stage for a variety of conditions, including diabetes. Yet, phytomedicines such as *Momordica Charantia Linn.* (Cucurbitaceae), have been found to increase skeletal muscle glucose uptake, and gluconeogenic enzymes which often are two important factors when considering the preceding pre-diabetic insulin resistance[3-5]. In fact, recent attempts have been made to elucidate the phytochemical properties which MC has, in relation to glycemia and hepatic functioning. Still obesity has ballooned into one of the most prevalent diseases of Western civilization [1, 2].

This review will focus on use of MC as a potential phytomedicine for preventing and/or treating insulin resistance, a key precursor associated with type II diabetes. This will be accomplished by first giving a brief review of the pathophysiology of insulin resistance, followed by animal, human and cell culture studies on MC. The following databases were consulted when choosing studies that were appropriate to this review: Medline, Cochrane Database, Scopus, Web of Science as well as Cochrane Database of Clinical Trials. Journal articles that were not from peer reviewed journals were not included in this review as critical data to support those particular studies were not sufficiently provided.

The Diabetic Dillema: Obesity and Insulin Resistance

In normal individuals, plasma glucose remains in a narrow range, i.e. between 4 and 7 mM, despite periods of feeding and fasting. This precise control is orchestrated by the balance between glucose absorption from the intestine, production by the liver and uptake and metabolism by peripheral tissues. Insulin acts as a primary regulator of blood glucose concentration by increasing glucose uptake in muscle and free fatty acid and inhibiting hepatic

glucose production. Insulin resistance, which can be defined as the reduced biological action of insulin, results in profound disruption of these processes [6]

The association of obesity with type II diabetes has been recognized for decades, and the major basis for that is the ability of obesity to engender insulin resistance, is a fundamental aspect of the etiology of type II diabetes [6]. Despite many details of the mechanisms by which the enlarged adipose tissue mass that defines obesity causes systemic insulin resistance remain unknown; *Figure 2* (Appendix) illustrates the main evidence supporting the link between increased adiposity and the development of insulin resistance. Increased adiposity leads to a greater appearance of free free fatty acidty acids (FFA) and glycerol within the blood stream. An increase in FFA and glycerol fluxes to the liver stimulates hepatic glucose production (HGP) [6]. At the skeletal muscle level, high FFA availability and/or reduced free fatty acidty acid oxidation capacity associated with obesity results in the progressive intramyocellular accumulation of triglycerides, which are likely associated with skeletal muscle insulin resistance [7]. Increased adiposity eventually leads to the deposit of adipose tissue in the liver. Typically, the liver functions as a major source for storage of glycogen, which can then be broken down to glucose in times when the body is in a sparing state, such as sleep. With an increase in free fatty acid storage in the liver and skeletal muscle, glycogen storage becomes impaired resulting in an increased plasma glucose concentration. To further complicate this issue the increases in rate of appearance of circulating glucose signals an effort within the body to maintain glycemic homeostasis. Specifically the pancreatic beta-cell is continuously signaled; by high levels of plasma glucose, which results in increased circulating insulin which functions to attenuate levels of plasma glucose. Skeletal muscle and adipose tissue cells are highly receptive to insulin. Yet if exposed continuously to insulin over a greater amount of time this relationship between signal

and receptor begins to diminish. It is this state which is referred to as insulin resistance, or the inability for insulin to either bind to its receptor. As a result, the disposal of glucose is far more difficult, as insulin must bind to its receptor to allow for the translocation protein Glut-4 to import glucose within the cell. Further consumption of high glycemic and high triacylglycerol foods furthers this state until the body slowly enters a diabetic state. At this point, the body resorts to a combination of free fatty acid and protein in order to meet its daily caloric needs for functioning, which can lead to muscle wasting and further ketometabolic acidosis and death if no intervention is taken.

A Bitter Sweet Option

Momordica Charantia Linn. (MC) (Cucurbataceae), commonly known in some cultures as Karela (India), Sorrow See (Caribbean), Ampalaya (Philippines) or Nigauri (Japan), Balsam Pear (North America) is a herbaceous vine which is found typically in sub-tropical climates [8, 9]. MC is currently grown in the Dominican Republic, California (USA), as well as in China, India, South/Central America and parts of Africa for commercial sale. Constituents of both the plant and leaves have been used widely in many natural medicinal preparations [9]. It is known to have a bitter taste associated with it, which for many makes it unpleasant to the palate.

Active Phytochemical Components

MC has many medicinal ingredients that have been isolated and acknowledged within the scientific community. Most studies have used extracts of the juice; which have yielded many medicinal compounds. Modern techniques in chemical extraction have yielded a host of

bioactive components including, β-momorcharin [10] a ribosomal inhibiting peptide, which has been reported to inhibit damage to RNA during the transcriptional phase of DNA production. Further, compounds such as Momordicin I and II and 5, 25 stigmastadienol which are triterpine based components, which have been associated with mimetic properties related to enzymes and glucocorticoid based hormones which are involved in glycemia. Finally a variety of triterpines have been found in MC [11]. As well, other compounds such as saponins (i.e. the glycoside portion of steroids, steroid alkaloids or triterpines found in plants, linked to medicinal qualities associated with cholestoral reduction) and triterpenoids have also been identified within the seeds of MC including, momordin Ic [12] and sitosterol, and decosahexanoic acid.

How these phytochemicals stimulate the uptake of glucose may lie in their impact on key hormones, phase enzymes, and organs in the body. It has been reported that a juice based extract upon further phytochemical extraction exhibited sulphonurea type activity [13]. Sulphonurea based drugs are used to treat insulin resistance in type II diabetic patients by acting to increase receptor sensitivity to insulin, which may have been disrupted in the initial stages of the pathology. As an example, the drug Metformin; which was isolated from the French lilac, has been associated with improvements in fasting hepatic insulin sensitivity and glucose clearance? Sulphonamides can also under insulinised conditions potentiate glucose disposal.

A ribosomal inhibiting peptide (RIP) known as β-momorcharin has also been identified in an ethanol based MC juice extract [10, 14]. Ribosomal inhibiting proteins reduce protein synthesis, which is critical to cell function and moreover cell death. This particular RIP protein has not been explored in diabetic models (*in-vitro* or in animals) and thus it would be necessary to explore this

idea further. This would be important in the pathology of type II diabetes, as for in many cases beta cell failure may occur due to necrosis. The necrosis could be the result of oxidative stress and in relation to obesity could be due to the release of pro-inflammatory cytokines such as tumor necrosis factor alpha upon excess adiposity. It should be mentioned that adipokines have only recently been researched with respect to MC [15, 16]. As well, this particular RIP would not directly be associated with any type of hypoglaecemic effect, but rather the protection of existing pancreatic beta cells against necroses or cellular oxidative damage which could impact beta-cell DNA.

Recently a class of saponins known as triterpenoid- glycosides were identified in MC (*Figure 2*) [17, 18]. These saponins have not yet been evaluated for effects in type II diabetes models but related saponins found in other plants such as those in ginseng, have revealed they decreased plasma blood glucose levels in patients that suffered from insulin resistance [19].

Saponins also have a potent effect on membrane permeability and receptor sensitivity. In obesity and type II diabetes, insulin receptors may be unreceptive thereby preventing insulin mediated glucose disposal into the cell [19]. Murakami et al. (2002) isolated a number of saponins and from MC which fall into the goyaglycoside or momordicoside families. Goyaglycoside-a through h were identified, along with goyasaponins I through III. As well, six varieties of momordicosides were identified and labeled accordingly Athrough F , along with gypsogenin, goyaprosaponin, and three oleanine type triterpene saponins [17]. These saponins have not been tested individually in *in-vitro* or in vivo studies, as per their recent discovery. Furthermore there is evidence that different varieties do have differing phytochemical constituents and overall content respectively [20, 21]. Therefore they are important candidates to evaluate, and warrant further study into their mechanism of action.

Studies on Animals: Promising Results

In recent studies, MC has been noted to be effective in streptozotocin (STZ) and alloxan treated rats. These rats are partially treated with streptozotocin or alloxan in order to mimic the intermediate state of beta cell failure incurred during type II diabetes. In animal studies Metformin® is normally administered in placebo controlled designs. It has been associated with several mechanisms linked to hepatic glucose production including, sensitization of the liver to glucose deposits, and down regulating gluconeogenesis [22].

In one such study MC was administered in a 50mg/kg dosage prepared from an alcohol extract to two groups of female wistar rats (n=6/group) over a period of three weeks. After eating a staple diet of rat pellets separate groups of rats were administered either tolbutamide or MC after an 18 hour fast. A third group of STZ induced diabetic rats were dosed with Metformin. Overall, a 10-15% decrease in plasma glucose was observed with the group of wistar rats who were administered tolbutamide, whereby after three hours MC elicited approximately 26-30 percent of what Metformin® would normally depress glucose by. However, that being said, insulin levels were not increased, as per normal response [23]. This would suggest that MC could be acting more so hepatically, in particular on down regulating hepatic glucose production.

Typically water and ethanolic based extracts of MC are used in *in-vivo* studies involving diabetically induced rats or mice. Sarkar et al (1996) administered three different dosages of MC juice respectively to male wistar rats (5.6%, 4.8%, 4.1% concentration of MC content), and found a significant impact on blood glucose levels [24]. The water based extraction that was prepared

(4.1% concentration of MC) was found to be the dosage that reversed alloxan induced hyperglycemia in rats with no toxicity to liver and kidneys for the 4 week experimental session. It was also determined that a 48% depression in plasma glucose occurred as compared to the oral hypoglycemic glibenclamide®. In this study, the number of rats per group was unknown, as for only a standard deviation was given in the results section.

Using a similar dosage (50, 100 and 200 mg/kg), in a 6 week study of male alloxan rats, MC was administered to separate groups of rats (Control, Diabetic) and showed a depression in plasma glucose of 15.4%, 18.7% and 22.9% in plasma glucose levels on days 40, 50 and 60 respectively ($n=8$, $p\leq 0.01$) [25]. Both alcohol and water based extractions were prepared and showed marked decreases in plasma glucose levels for respective dosages. Skeletal muscle glycogen content and hepatic glucokinase levels were observed to be partially restored as well as hexokinase, glucose-6-phosphate and phosphofructokinase (PFK). PFK is known as a rate limiting enzyme in the process of glycolysis, which is impaired in type II diabetes [39].

Shibib et al. (1993) found that MC contributed to the depression of gluconeogenic enzymes glucose-6-phosphatase and fructose-1-6-biphosphatase but elevated the liver enzyme glucose-6-phosphate dehydrogenase (G6PD) in control and MC administered STZ rats ($n=4$, $p\leq 0.01$) [26]. G6PD has been identified as being involved in a separate pentose-5 pathway, which is a tertiary pathway for glucose production. Furthermore depression of key gluconeogenic enzymes would give further evidence that MC impacts the body hepatically.

Ahmed et al. (1998) found that STZ induced diabetic mice, when administered MC juice extract (10ml/kg body weight) were observed to have a decrease in islet destruction as compared

to the placebo group. Furthermore repair of existing damaged islets was also observed [27, 28]. This finding adds to the evidence that MC may act as an anti-oxidant and further promote enzymatic response associated with glucose uptake and disposal.

Although results seem promising in the animal model with respect to MC, one study in particular did not find an effect with respect to administration of a set dose. Karunanayake et al. (1990) found that administering both acute and cumulative doses of MC juice (10ml/kg) to STZ induced diabetic rats had no effect on plasma glucose clearance. A control group and two experimental groups were used to determine the effect of dose responsiveness. After being administered the Oral Glucose tolerance test, either acutely or after a 30 day exposure period, a total of three blood samples were taken to measure the dose responsive curves. No significant effect was found between the control and experimental groups of rats. [29]

Emerging Evidence: Energy Expenditure, Adiposity and Appetite

In a recent study, male alloxan rats were supplemented with MC, with each group being fed a standardized diet for a period of 4 week period. An increase in overall energy expenditure was observed in the experimental group versus that of the corresponding placebo group. In the same group of rats, body composition changed, whereby the experimental group fed MC had less visceral adipose tissue (VAT) deposit. Therefore, this result may reveal a link between MC consumption and the potential to inhibit the onset of obesity. This trial also was much longer than those previously performed, and occurred under strict laboratory setting, with a high level of statistical power [16, 30].

In addition the study of appetite is crucial to understanding more about obesity. It has been noted that variables such as gastric transient time may play a key role in appetite. Momordin Ic has been found to decrease gastric transient time in rats. This pungent phytochemical can be found in capsaicin. Momordin Ic typically found in the seeds of MC has been found to decrease gastric emptying [12, 31, 32]. Decreased gastric transient time has been found to have a positive effect on satiety. Gastric transient time if decreased would slow the movement of food within the intestinal track. As a result if the breakdown breakdown of carbohydrates and foodstuffs would occur at a slower rate, which could potentially slow the rate of appearance of glucose within the blood stream. However, gastric transient time has not been a priority in studies related to MC, and is very difficult to conduct. Further study should track this component, as hunger and satiety are key components related to weight gain and adiposity.

In-Vitro Based Evidence

Recently researchers have used L6 myotubes in order to examine whether MC may simulate glucose uptake in muscle cells [33]. Two separate extracts were prepared (Extract A) a lyophylised form, and the other which was chloroform based (Extract B). After 1h of incubation, insulin-stimulated deoxyglucose uptake significantly increased within the myotubes grown in Extract A as compared to control. Yet the results indicated that this was not necessarily based on the augmentation of dosage. Rather three separate administrations of the 5μg/ml extract-A actually promoted the uptake of deoxyglucose greater than corresponding 10 μg/ml dosages respectively. This evidence indicates that higher pharmacological dosing may actually impede the functioning of MC on skeletal muscle in rats. To our knowledge this response based study has not been explored or yet seen in human based studies.

Since glucose uptake was observed in L6 cells, further interest has peaked regarding absorptive features of MC. In an *In-vivo/In-vitro* study Ahmed et al. (2004) investigated the post absorptive pathway of MC by examining brush border vesicles from the jejunum of STZ rats who were administered MC for a 10 week period [27]. Concurrently, L6 myotubes were also utilized, to determine if ^{14}C-D glucose uptake would occur. The results showed that diabetic rats gained less weight and had higher glucose values than healthy rats ($p \leq 0.001$). Yet when administered MC at a concentration of $5\mu g\ ml^{-1}$ blood glucose values declined over the length of the experiment ($p<0.05$), as compared to the diabetic group ($p<0.001$). Weight stayed relatively the same, yet plasma insulin and the number of insulin positive cells in the diabetic group declined ($p<0.05$). Conversely the MC treatment group both increased the number of insulin positive cells and plasma insulin concentration ($p<0.05$). What is worth mentioning is that these values were significantly lower than those of the healthy untreated control group ($p<0.05$). Thus MC seems to promote brush border intestinal glucose uptake, and promote glucose uptake into skeletal muscle.

A Clinical Approach: Investigation in Humans

Research into the validity of the usage of MC on humans began in the latter half of the 20th century. Leatherdale et al. (1981) were one of the first to study the effects of MC on humans, and found that this fruit at a dosage of 50ml (juice) lowered serum glucose considerably in a group type II diabetic men ($n=6$, $p \leq 0.05$) [34]. This study involved consuming bitter melon in the juice (50ml), after administration 50g oral glucose tolerance test. All participants in the study were diabetic, and were taking some form of oral hypoglycemic including tolbutamine, glimenclamide or glymidine respectively. The participants were taken off the oral hypoglycemic

for a period of 48 hours prior to the experimental condition. While this would allow for medications to clear the body, it also is dangerous for health to say the least.

Clinically a high glucose load is administered to patients in order to examine insulin and glucose response to determine if diabetes is present. This high glycemic load is known as an oral glucose tolerance test (OGTT). Welihinda and colleagues (1986) demonstrated that MC juice (100ml) lowered plasma glucose after the administration of an OGTT (50g solution), in 73% of diabetic patients receiving it ($n=18$, $p \leq 0.05$). Water (100ml) was given as the placebo dose to both an experimental ($n=13$) and control group ($n=5$), followed a day after by administering MC. The control group was administered the MC 30 minutes before the OGTT [35]. A placebo was used in this experiment yet its lack of similarity to the taste and texture of MC was not evaluated, and further could have biased the study.

Since MC has compounds which may aid in the uptake of glucose within cells, the issue of co-commitant administration of MC with oral hypoglycemic agents requires much prudence. For instance Ahmed et al. (2002) administered a dried extract of MC to diabetic participants who were taking a prescribed oral hypoglycaemic. These patients were administered (body weight of individual in (mg) * 2) a dosage, which was determined by the author of the study [36]. Ultimately within the human clinical studies involving human participants there are a multitude of issues which could call into question the validity of the results. Standardization of the clinically administered oral glucose tolerance test (OGTT), inclusion criteria and dosage are always of concern.

In Germany another study was undertaken, whereby 500 mg capsule of freeze dried MC was given in conjunct with a prescribed oral hypoglaecemic. This study was conducted in a randomized control design with 47 patients participating in the study. The results were encouraging as patients who took the standardized 500mg extract of MC reported a positive effect on the depression of plasma glucose levels ($p<0.01$). The dosage of the prescription medication was lowered in order to compensate for the addition of MC. This was due to the additional hypoglaecemic effect which MC exhibits [37].

Currently many of the researched extracts of MC given to patients have not been phytochemically evaluated. Dose responsive models should attempt to establish product contents in terms of phytochemical constituents. As well a placebo controlled design would prevent any cases of bias during experiments. These two factors would allow researchers to better identify potential mechanisms associated with respect to glucose disposal in human models.

Conclusion: Where are Standardization, Quantification and Effectiveness?

To date much study has been done in relation to MC and rats. In particular these studies have focused on alloxan and STZ induced diabetic rats. What therefore may be problematic is that in order to mimic the diabetic state, these animals are treated with specific toxic drugs. While the treatment used to induce this state may accomplish their goal, other organs may be affected and must be taken into consideration.

Taken together, some, albeit limited, data suggest beneficial effects of MC in diabetic humans. However, further information in randomized placebo-controlled trials is needed. To our

knowledge, few studies have attempted to elucidate the acute effect of MC on glucose homeostasis in humans. As well the effects of MC on glucose control in obese men has thus far not been investigated which could allow the research community to understand whether MC could be used at an earlier stage to prevent diabetes? Obese individuals have high levels of fasting and postprandial glucose [38], which could render them more susceptible to develop type II diabetes. Thus research with overweight/obese individuals could provide some information about the potential impact of MC on circulating adipokines, and adipose tissue-derived hormones regulating systemic insulin sensitivity. Energy expenditure is also a major component in the pathogenesis obesity. Thus, future research should also focus on this parameter in relation to MC in humans.

One issue that does remain problematic within clinical studies of MC remains the standardization of dosage as well as the phytochemical identities of the extracts themselves. Dosage as well as quantities of phytosterols and related constituents should be taken into consideration when performing such research. Thus, the issue should be addressed in both clinical animal and human based studies respectively. Further to this, studies in humans have not used a suitable comparator such as sulphonylurea drugs versus MC in a double blind repeated measures design.

Understanding how *Momordica Charantia L* influences glycemic control and body weight regulation may have important implications for the potential use of MC as a supplement for preventive and curative interventions in obesity and diabetes. Currently problematic issues concerning supplements exist, in terms of the evaluation of such substances and their validity in their utilization for the treatment of obesity. As well phytochemical concentration of compounds

found in *Momordica Charantia L.*, may put consumers at risk if taken improperly. In particular, the effects of phytochemicals on human physiology and drug nutrient interactions need to be studied further.

Objectives & Hypotheses

To our knowledge, there has been no placebo-controlled attempt to elucidate the acute effect of MC on glycemia, energy metabolism and appetite in healthy men. While MC has been experimented in a human model, it has been done so solely in diabetic individuals. As mentioned, previous studies have shown that MC has an acute positive effect on glycemic regulation in diabetics. Therefore our primary objective was to examine the acute effects of MC on fasting and postprandial glycemia in healthy overweight men. We predicted that MC would lower fasting and postprandial glycemia. Our second objective was to determine the acute effects of MC on energy expenditure. Limited information from rat studies have indicated that overall energy expenditure increased when MC was administered with a standardized diet (Chen et al). We predicted that MC will increase overall acute energy expenditure. Our third objective was to explore the acute effects of MC on appetite. Since gastric transient time has been shown to decrease in rats who were administered momordin Ic, it was our hypothesis that this decrease in gastric transient time would slow the rate of appearance of glucose within the bloodstream, thus leading to a delayed response for appetite. Thus we predicted that MC would acutely decrease appetite. This factor has never been examined in humans.

Assumptions, Delimitations and Limitations

The present study assumed that the participants answered honestly to the pre-screening questions and criteria, and that they did not exhibit any metabolic diseases or dysfunction. It was also assumed that the selected volunteers followed the pre-experimental protocols such as fasting before hand for a period of 12 hours prior to the experiment, and refraining from any physical activity or alcohol consumption before the respective date of experiment. It was also assumed that indirect measurement of substrate oxidation by expired gas analysis is a valid measurement of whole body oxidation. The measurement of substrate was limited to the analysis for expired gas for determining whole-body oxidation of substrate, as opposed to measurements using tracer technology to be able to determine the location of substrate oxidation and glucose uptake respectively. It was assumed that the quality of extract produced in the biopharmaceutical science laboratories were of utmost high quality from MC fruits which were semi-ripe. Finally, the blood analysis techniques employed by laboratories were assumed valid for the measurement of plasma glucose and insulin levels.

In order to answer the research questions which were proposed in this study the experimental conditions were delimited to the morning, post absorptive period between 7:30 am to 12:30pm respectively. Within this timeframe, the oral glucose time period was limited to three hours. In order to minimize inter-individual variance in metabolism, the selection for participants was delimited to overweight healthy men whose body mass indices met the criteria for overweight men ($\geq$25 BMI) (BMI = body mass(kg)/ height $(m)^2$) in accordance to the WHO (1998). In addition, the ages of participants were delimited to the range of 18 to 55 years of age.

Significance of the Study:

The present study attempted to provide new information in the area of natural health products, diabetes and insulin resistance by examining the effect of a dose dependant response to MC in healthy overweight men. To date many studies have focused solely on the diabetic model, whereby a randomized placebo control design has not been used. Further to this substrate oxidation and appetite in humans has not been investigated after consumption of dosages of MC. Thus our study attempted to explore not only glycemic factors associated with MC, but substrate oxidation and appetite using a randomized placebo control design for administration of MC.

CHAPTER II

Original Article: No Effect of Oral Administration of Momordica Charantia Linn., on Glycemia, Energy Expenditure and Appetite: A Pilot Study in Overweight Men.

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Abstract

Phytomedicines are currently being researched for the treatment of various pathologies, including those involving carbohydrate and lipid metabolism. *Momordica Charantia Linn* Cucurbitaceae (MC), otherwise known as ``Bitter Melon``, Nigauri (Japan), Sorrow See (Caribbean) and Karela (India), has been used to treat glycemic impairment in relation to diabetes for centuries. The objective of this study was to determine the acute effect of MC on post-prandial glucose levels, energy expenditure/ fuel mixture and appetite in normal body weight individuals. Five healthy men (34 ± 14 SD y; 27 ± 0.34 SD kg/m^2 BMI) were supplemented on three randomized conditions where 1) no MC (Ctl), 2) 50 mg/kg g of glucose (MC50) or 3) 100 mg/kg of freeze dried MC (MC100) were given orally prior to a 75 g oral glucose tolerance test (OGTT). Plasma glucose and insulin levels were measured before and during the OGTT. Energy expenditure as well as carbohydrate and lipid oxidation rates were measured by indirect calorimetry. Finally, visual analogue scales were used to rate appetite. Plasma glucose and insulin levels significantly increased during the OGTT ($P < 0.05$) but no significant difference was observed between experimental conditions. Energy expenditure did not change over time, regardless of the experimental conditions. During the OGTT, a shift from lipid to carbohydrate oxidation was observed, regardless of the experimental conditions. Finally, no treatment effect was found for appetite scores throughout any of the experimental sessions. These results suggest that from an acute standpoint MC does not affect plasma glucose and insulin levels, energy expenditure, substrate mixture and appetite scores following an oral glucose load in healthy men.

Keywords: Phytomedicine, Obesity, *Momordica Charantia L*inn, Energy Expenditure, Appetite, Humans, Visual Analogue Scale, Hunger.

Introduction

A medicinal plant that has putative effects on reducing complications associated with the onset of diabetes is *Momordica Charantia Linn.* (MC). It is a member of the Cucurbitaceae family (Gourd), and is grown mainly in sub-tropical climates in countries such as Thailand, India and Jamaica (2, 3). MC juice has many medicinal properties including lowering insulin levels in type 2 diabetic rats (4-14).

Potential mechanisms by which MC may aid in regulating glycemia include *In vivo* studies reveal MC's action as an insulin secretagogue (15, 16) and may be responsible for increases specific phase enzymes in the liver which may be impeded during insulin resistance (17, 18, 18-20). Furthermore, chemical analysis has also revealed that MC has many phytochemicals which may delay gastric transient time, and function to protect damage to key tissues (pancreas, liver) involved in the regulation of glucose metabolism (19, 21-29)

Clinical experimentation with MC was first documented by Leatherdale et al. (1981) whereby it was found that MC lowered serum glucose considerably (30). As well, Welhinda and colleagues (1986) demonstrated that MC decreased glucose levels in 73% of patients receiving it (31). Further clinical studies by Ahmad et al (1999) and Akhtar (2003) also revealed that MC juice lead to a significant reduction of both fasting and postprandial glycemia in diabetic participants (5, 32). Conversely one study found little or no effect of MC administered in the juice form which is normally consumed in ethanol based extract (33). It is important to mention that some studies have used the juice, while others have used a dried powder form of the fruit. A placebo control design was not used in these clinical studies. Thus, these clinical studies lacked a randomized placebo control design, which would therefore put into question overall efficacy of results (34).

Animal studies reported that chronic MC supplementation not only improved glucose tolerance and lowered plasma glucose, but also reduced adiposity in rats fed a high-free fatty acid

diet (56). These observations were not attributable to decrease in energy intake or free fatty acid absorption, suggesting then effects on energy metabolism. The effects of MC on energy expenditure and substrate oxidation as well as appetite levels have never been characterized in humans.

Taken together, some, albeit limited, data suggest beneficial effects of MC in diabetic individuals. To our knowledge, the proposed study represents the first randomized placebo-controlled attempt to elucidate the acute effect of MC on glycemia, energy metabolism and appetite in healthy men. Our primary objective was to examine the acute effects of MC on fasting and postprandial glycemia in men. We predicted that MC would improve fasting and postprandial glycemia. Our second objective was to determine the acute effects of MC on energy expenditure. We predicted that MC increases overall acute energy expenditure. Our third objective was to explore the acute effects of MC on appetite. We predicted that MC would acutely decrease appetite.

Methodology

Participants and Recruitment

Participants were recruited at random, using information and pamphlets posted at medical and community health facilities. Five men gave their written consent to participate in this study that was approved by the Research and Ethics Board of the University of Ottawa. All subjects were healthy, as determined by a medical history questionnaire, and none of them were smokers. The average age of participants was 34 years (±14 years) (mean ± SD).

Study Design

A randomized, blind, placebo-controlled, cross-over laboratory study was implemented. Interested participants were initially screened via telephone to determine if they meet the criteria of the study. Eligible participants were then asked to come to the laboratory for a pre-screening session

Prescreening Session:

Participants were asked to arrive at 8:00am EST. They were asked to change into a hospital gown provided to them, and remove all metal substances they were wearing as they could interfere with the dual x-ray absorptiometer (DEXA). They were then asked to answer a lifestyle questionnaire (Appendix). If the participants were found to have complied with all inclusion factors they were then asked to proceed to the DEXA room to have anthropometric measurements taken. After the anthropometric measurements were taken, they were then scheduled for their first testing session.

Anthropometric Measurements

Body weight was determined with a standard beam scale (HR-100; BWB-800AS, Tanita Corporation, Arlington Heights, IL., USA), whereas height and waist circumference were measured with a tape. Body weight was measured after voiding, while clothed in a hospital gown

and after having removed all accessories (watches, bracelets, chains, eye glasses, etc.) Height was measured with the participant's bare feet together, with heals, buttocks, back, and head against the wall, and following a normal inspiration. Waist circumference was measured directly on the skin, in duplicate (and averaged), at the mid-point between the last floating rib and the top of the iliac crest. Body composition was determined using dual-photon x-ray absorptiometry (DEXA) which measures fat-free mass, fat mass and total body fat percentage (Lunar Prodigy, General Electric, Madison, WI, USA). Participants were required to lie on the measuring unit's examination table, in hospital garments (with all metal objects removed); while a low-intensity x-ray scanned the entire surface area of the body. The measurement took approximately 15 minutes to complete and the radiation associated with it is less than 0.5 millirem. Coefficient of variation and correlation for percent body fat measured with the DEXA in the 5 participants tested in our laboratory were 1.8 % and $r = 0.99$, respectively. (see Table 1)

Experimental Protocol

Eligible subjects underwent the following three experimental conditions in a randomized order: placebo, MC 50 mg/kg body weight and MC 100 mg/kg body weight freeze dried juice. The latter dosages of MC covered the approximate amount of MC extract previously given in the literature (8-10). Each of the experimental conditions was separated by 1-week. No adverse effects were reported by the participants throughout the study.

During each experimental session (see Figure 1), the participants were asked to fast for a period of 12 hours prior to arriving (7:50 am) at the laboratory. Participants were also asked to refrain from any vigorous exercise 48 hours prior to experimental sessions and to refrain from consuming alcohol on the day prior to all experiments. Participants kept a record of their regular eating patterns in a 3-day journal before their first experimental session and maintain the same eating pattern prior to the 2 remaining experimental sessions. Once these recommendations respected, participants were then inserted with a catheter into the antecubital vein for the collection of blood samples during the experiment. A separate saline solution was administered to the participant between sample collections. They were then instructed to lay down for a 15-minute resting period which was followed by a 30-minute resting metabolic rate (RMR) measurement by indirect calorimetery (Delta Trac, GE Healthcare) via a canopy system.

Following the RMR the participant was then asked to ingest the dosage of the supplement (8:45 am). A 75 gram oral glucose tolerance solution was given to the participant to help with the ingestion of the pills, and provide a high glycemic load to the participant. The subject was required to wait approximately 15 minutes after ingestion in order to ensure the absorption of the supplement. Blood samples during the OGTT were collected in tubes through the catheter at, 0, 30, 60, 90 and 120, 150, 180 minutes. Plasma insulin and glucose, concentrations were determined at each time point. Energy expenditure was also measured be along the OGTT by indirect calorimetery by sampling expired air 15 min every 15-minute period.

Insulin and Glucose Measurements

Blood samples were centrifuged at 3500 r/min and plasma was stored at -80°C for future assays. Plasma glucose concentrations were assayed using spectrophotometric analysis after conversion of glucose to glucose 6-phosphate by hexokinase. Laboratory-grade reagents (Sigma-Aldrich Canada Ltd., Oakville, Ont; Fisher Sientific Ltd., Nepean Ont.) were used for preparing a standard hexokinase reaction, and after 30 min incubation of prepared samples at room temperature, spectrophotometric analysis of resultant NADH light absorbance was performed in duplicate using a Synergy HT Series Multi-Detection Reader (Bio-Tek Instruments Inc., Highland Park, Winoosi, Vt.), with absorbance readings of 340 nm wavelength emissions. Samples collected from the three experimental sessions were analysed on the same plate. The intrassay coefficient of variation for glucose analyses was 3.4%. A 2-site ELISA immunoassay using 2 monoclonal antibodies (LINCO Research, St-Louis, Mo.) was used to measure plasma insulin levels with intraassay coefficient of variation of 3.5%.

Measurement of Energy Expenditure and Substrate Metabolism

The indirect calorimetry system (Deltatrac II, SensorMedics, Yorba Linda, CA, USA) was calibrated against 95%O_2/ 5%CO_2 reference gas before each test. After a 20-minute rest period in the supine position, a measurement of resting energy expenditure (REE) was performed. In order to obtain REE measurements, a Plexiglas hood was placed over the participant's head through which fresh air was drawn. The expired air was sampled and analyzed for oxygen and carbon

dioxide averaged per minute and determined for 30 minutes. The Deltatrac II system has a coefficient of variation and correlation for VO_2 measurement in the 5 participants tested in our laboratory of 0.97 % and r = 0.95, respectively. From respiratory measurements, total free fatty acid and carbohydrate oxidation rates were calculated using the non-protein respiratory quotient (57):

Carbohydrate oxidation rate (g/min) = 4.585VCO_2 (l/min) - 3.226VO_2 (l/min)
Lipid oxidation rate (g/min) = 1.695 VCO_2 (l/min) - 1.701 VO_2 (l/min)

Appetite:

Visual analogue scales (VAS) were used to assess four parameters with respect to appetite measurements: Desire to Eat, Level of Fullness, Hunger and Prospective Food Consumption (PFC). Appetite ratings were measured before and every 15 min the OGTT. The scales were designed such that a specific line on the page would range from weak to strong. A pen mark was stroked along the line (150 mm), indicating to what level the participant felt with respect to the statement (Hill and Blundell 1986). The scales used were measured in millimeters, and subjects were asked for further verification of meaning if they did not understand the context.

Preparation of Extract

Fresh Bitter Melon (Origin: Dominican Republic) was obtained from a local supermarket chain (Fresh Fruit Company Ltd.), and brought to the Center for Research in Biopharmaceutical and Biotechnology (CRBB), at the University of Ottawa. It was then washed with a food grade detergent to remove any pesticide residue. The fruit was then weighed before being cut into four long pieces, whereby the seeds were removed, and placed in a food juicing machine (Hamilton Beach ®). Juice was collected in a container, whereby a lid was placed on top, and left in a refrigerator (4°C) for a period of 1 hour. After many of the air bubbles disappeared, it was then placed in a freezer (-20°C) overnight. A freeze drier was then activated the following morning (-60°C), whereby the frozen juice was placed. It was left for a period of 3-4 days, depending on amount of juice collected. After it was determined to be dry, it was weighed. A sample of the

plant freeze dried plant material was deposited as a voucher in the University of Ottawa herbarium.

The pills for administration for participants were compounded in the food grade laboratories of the CRBB. This was done via a pill encapsulator, and each pill was weighed such that the number of pills for the 50mg/kg dosage was the same as that for the placebo and 100mg/kg dose. Quinine (0.0001g/mg), which has a bitter taste, was added to the placebo to ensure that participants were unable to differentiate treatments by taste, of which treatments included a placebo, 50mg/kg body weight or 100mg/kg body weight of freeze dried MC. The placebo for this trial was cellulose because it has no known activating properties on pancreatic function (49) Pills were then placed in Falcon sterilized tubes and placed in a freezer at -20°.

Statistical Analysis

The software program SPSS version 12 for windows (SPSS Inc. Chicago, IL, USA) was used for the data analysis. Data was first collected in Microsoft Excel, whereby it was then transferred to SPSS. A one way ANOVA for repeated measures was performed with two within subject's parameters (effects of experimental conditions, i.e. No MC, MC50 and MC100, and effects of time). Alpha was set at 0.05

Results

Anthropometric Measurements

As shown in Table 1. (Appendix), participants did not show any significant variation in body weight waist circumference, percentage body fat, or waist girth while taking part in the three experimental conditions (Control, MC50mg/kg, MC100mg/kg).

Plasma glucose and insulin levels

Changes in plasma glucose and insulin levels are presented in Figure 2. (Appendix) In the fasted state, plasma glucose and insulin levels were not different between conditions. Plasma glucose and insulin levels significantly increased during the OGTT ($P < 0.05$) but no significant difference was observed between experimental conditions. (see Figure 2)

Energy Expenditure and Substrate Utilization

Figure 3 illustrates the changes in energy expenditure (kJ/min) and substrate utilization before and during the OGTT while participants were supplemented with no MC, 50 mg/kg, or 100mg/kg. Energy expenditure was not affected by the experimental conditions. During the OGTT, a shift from lipid to carbohydrate oxidation was observed, regardless of the experimental conditions.

Appetite

Visual analogue scales measurements are illustrated in Figure 4. The mean appetite score for the desire to eat, hunger and prospective food consumption increased significantly over time ($P < 0.05$) but no difference was noted between experimental conditions. No change in the mean appetite scores for fullness was observed across time or experimental conditions.

Discussion

The objectives of this study were to examine the acute effects of specified dosages of *Mormordica Charantia Linn.* on specific factors such as glucose and insulin concentration, energy expenditure and finally appetite/satiety parameters. The results of this study suggest that an acute administration of MC does not affect: 1) glucose/insulin levels; 2) energy expenditure and fuel mixture and 3) appetite scores following an oral glucose tolerance tests.

In humans it has been observed that acute administration of MC stimulates an insulin secretagogue response (30-32) Unfortunately to date all studies with humans have been done in a diabetic model, which cannot validate the use of MC for patients who are healthy/overweight. This study was undertaken in order to reveal the effects of MC administration on healthy/overweight humans, as the problematic issue of weight control and diabetes in our population has become one of great significance. To further expand, this was the first time a randomized placebo control design was used in this cohort of participants. Our results indicated that neither a large (100mg/kg) nor small (50mg/kg) dose administration of MC administered to overweight patients had a significant effect on blood glucose or insulin concentration over the three hour period following the OGTT than that of placebo. This is in contrast to current literature which has shown a marked decrease in blood glucose concentration in diabetics (27, 34, 35, 36) In most studies the administration of MC was administered orally with no placebo to control potential biases. Furthermore, the type of dose administered differed between studies. For instance Leatherdale (34) Akhtar (36) and Ahmed (35) all administered juice orally; with no placebo, rather than a freeze dried encapsulated version in this study.

Chronic MC supplementation has been shown to reduce adiposity in rats fed a high-free fatty acid diet without altering energy intake and free fatty acid absorption (56) then suggesting an effect of MC on energy metabolism. To our knowledge, the present study is the first to measure energy expenditure and substrate oxidation rates following acute ingestion of MC in humans. Our results indicate that a single supplementation of MC does not stimulate energy expenditure and is not accompanied by any change in fuel (CHO and lipid) oxidation rates over a 3 hour period following an OGTT in men. One mechanism recently put forward whereby chronic supplementation (7 week) of MC slows weight gain in rats involves an increased of the sympathetic tone as measured by elevated circulating cathecolamines (56). Although our results

are not concordant with those of the previous study, this does not rule out that MC may increase energy metabolism only after a longer supplementation period in humans.

Appetite and hunger were hypothesized to decrease as dosage of MC increased. There were multiple reasons why this hypothesis was postulated. Evidence indicated that the administration of MC can influence the absorption of glucose in the brush border vesicles of the intestinal tract via increased action of renal disaccharidase (58). Another research group indicated that *in-vitro* administration of MC in a dose dependent manner resulted in primary inhibition of glucose uptake in rat everted gut sacs (59, 60). Theoretically MC could allow for decreased gastric transient time which would hypothetically allow for a lesser rate of appearance of glucose. This evidence is speculative and thus further clinical evidence *in-vivo* must be used to validate such a claim. Evidence in human models suggests that a delayed gastric time could inhibit appetite by means of less pronounced release of insulin or intestinal incretins (61, 62). This combined with *in-vitro* evidence for the activation of sympathetic nerves with the active ingredient Momordin Ic was what had initially led to the hypothesis that short term administration of MC would inhibit appetite (63). However, this was not the case as results from the visual analogue scales for each respective question proved to be statistically insignificant ($p<0.05$). Much of the evidence in evaluating appetite and MC was based on *in-vitro* experimentation as no human study to date has evaluated appetite as a parameter in a study.

Conclusion

The results of this study show that acute administration of MC does not affect glycemia, energy metabolism and appetite levels in healthy overweight men. This was the first study to date which experimented with this subset of participants. As such, further study, in the form of a long term clinical trial on humans should occur, whereby more factors could be observed if changes or absorption were dependent on time and length of administration.

CHAPTER III

Perspectives

This experiment was the first to be performed on overweight healthy individuals. It has also been the first to use a randomized control design with placebo. Scientifically steps were followed in order to endure that the trial would run smoothly, however, since we are dealing with human participants this can prove rather difficult. Issues such as participant dropout, and further rigorous screening criteria did prove to be a challenge but nonetheless this trial managed to recruit healthy overweight men.

Since this was a new endeavor there were some problematic issues that came to light during the course of this experimental trial. As the trial commenced one problematic issue emerged as another paper by Chen et al. (2005) suggested that the dosages administered in the rat models previously may not be effective in an obese model per se (47-49) . Within that particular study it was observed that a dose of 1.2 g/kg did illicit a statistically significant effect ($p<0.001$) on glycemic response, and other related parameters. As well, this study administered MC for a period of 4-6 weeks respectively, and fed rats a relatively high caloric diet, with a greater ratio of lipids to carbohydrates. This type of diet would mirror the current human situation, which is occurring in populations globally.

Therefore, if this experiment were to be conducted again, a longer term intervention would probably have yielded a different result, as Chen et al., observed the importance of daily administration for a period of greater than 4 weeks to achieve a greater significant effect on plasma glucose lowering post-prandially. Further, the participants chosen for this trial were healthy normal/overweight individuals. MC may have elicited a different response in a population such as pre-diabetics (insulin resistance overweight participants) than in the population which was experimented with in this protocol. Thus in a further study with MC the

use of a pre-diabetic obese model of humans would be preferable, as it is in this group where glucose homeostasis is impaired, and any potential effect of MC could be observed more readily. Thus dosage and participant parameters must be prudently observed and implemented in future studies with MC in order to ensure further reliability of a randomized control design which was used in this protocol.

Another problem involving standardization of dosage for this particular product and other NHP's is complex, but should be addressed in future trials. Standardization would refer to the amount of active ingredient present that might be involved with elucidation of a desired response. For example in the plant American Ginseng Panax, ginsenosides have been associated with increased insulin sensitivity in patients with diabetes (50). On the other hand certain dosages of Panax have been experimented with certain ginsenosides removed (51, 52). These dosages seemed not to produce a post-prandial effect as compared to the whole mixtures (57). A standardized dose of MC with a certain percentage of desired phytochemicals could allow researchers to observe particular response in relation to glycemia. What is problematic is the recent discovery of glycosides, and saponins in MC. For instance, structures of goyaglycoside, 5, 25 stigmastadienol, and Momordicine II were only determined within the last 7 years (24, 27, 53). Standards are not available for HPLS analysis of plant material as yet.

The synthesis of such molecules is often complex, and has not been. Most have been isolated via column chromatography or reverse phase HPLC analysis. In any case the action of MC has a known pathway, which impacts both insulin secretion and potential receptor membrane permeability. The activation of pancreatic beta cells, to secrete insulin acts such as to illicit a secretagogue type function. Saponins have a known biological action involving to improved

membrane sensitivity to insulin (54) Further testing could attempt to extract a particular component such as Momordicine I or Momordin Ic, and encapsulate this particular component to see what its specific action is in the body.

Future studies are needed to examine how MC phytochemicals was absorbed or for that matter tissue distribution and absorption. This could be accomplished using recently developed stable radioactive isotopes could allow researchers to be able to monitor glucose fluctuations in the body and further to collect protein content via urine samples, which would further allow the researchers to explore the relationship between MC administration and its action on the glucose metabolism pathway in the body.

Finally, one aspect of the study which could have been added was a buffet test to determine food preference, and hunger post-prandially, to further understand if MC could act as an appetite suppressant. This would involve a carefully selected diet with calculated values for each particular item that would be available to participants after the experiment would be concluded. This may allow researchers to understand food preference, after nutraceutical intervention, and further determine if in fact certain foods such as MC allow for appetite suppression.

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APPENDIX I: TABLES

Table 1: Anthropometric Measurements of Participants (n=5)

	Prescreen	Placebo	MC 50mg/kg	MC 100mg/kg
Age (years)	34 (+-14)	-	-	-
Weight (kg)	87.1 (+- 11.02)	87.1	87.5 (+- 11.23)	87.3 (+-11.00)
BMI (kg/m^2)	27 (+-3.31)	27	27.5 (+-3.09)	27 (+-.3.38)
% Body Free fatty acid	25 (+- 6.14)	-	-	-
% Free fatty acid Mass	26.2 (+-7.24)	-	-	-
% Free fatty acid-Free Mass	70.0 (+-6.68)	-	-	-
Waist circumference (cm)	86.36' (+-11.94)	86.3	85.09 (+-9.62)	86.49' (+-10.54)

APPENDIX II: FIGURES

Article I Figures

Figure 1. Effects of enlarged adipose tissue mass on systemic insulin resistance.

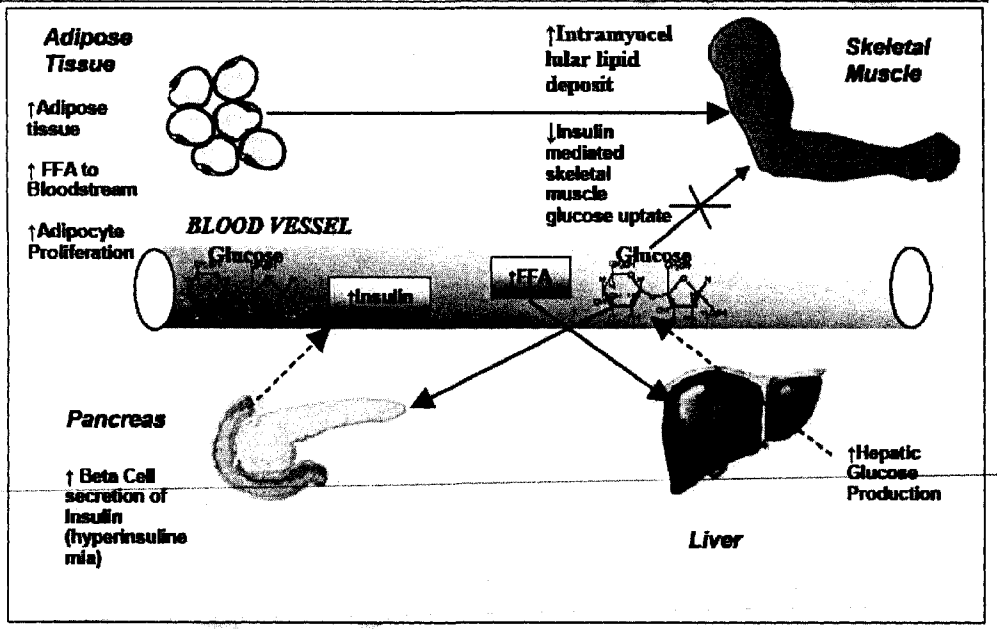
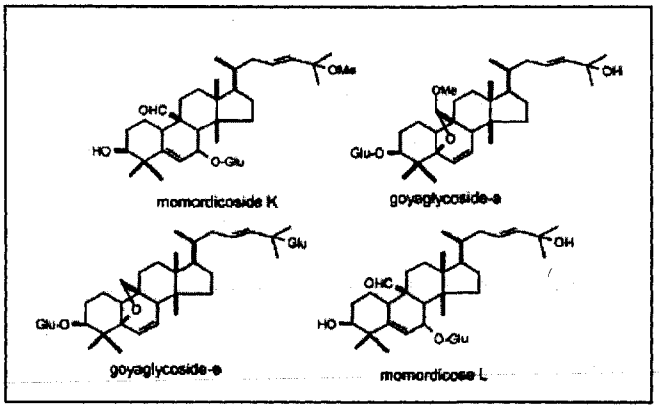


Figure 1: Description: Obesity augments levels of plasma free triacyl-glycerolty acids which flux to the liver stimulating hepatic glucose production; increased adiposity is also associated with high deposits of triglycerides within skeletal muscles, which has been associated with a reduction in skeletal muscle insulin mediated



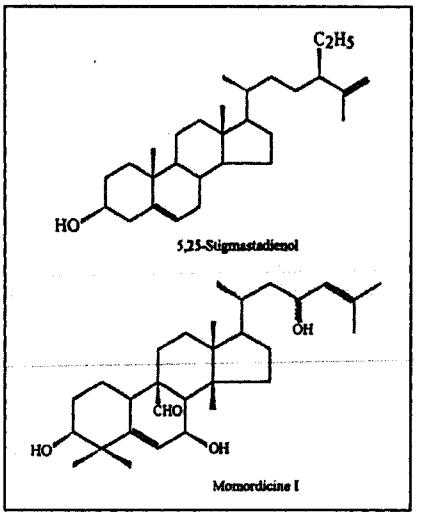
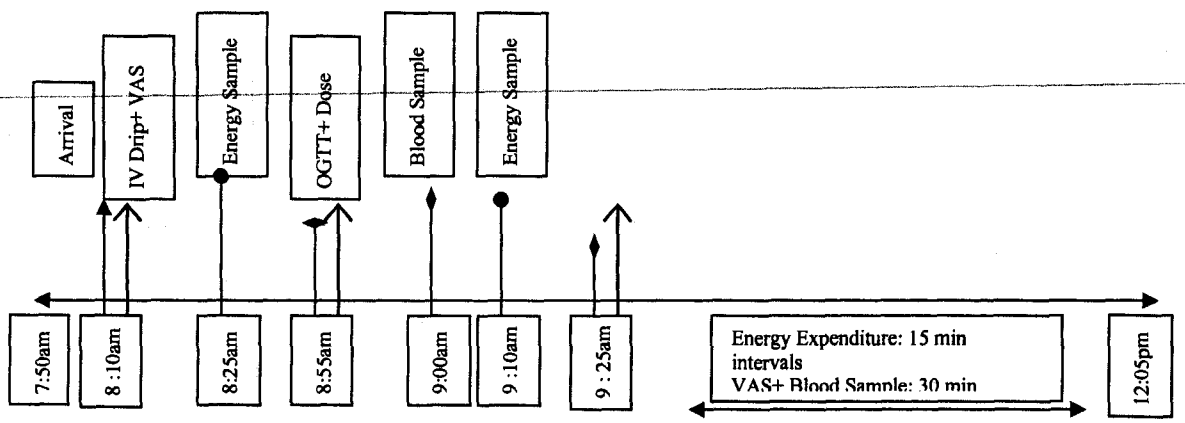


Figure 2. Isolated extracts from Momordica Charantia Linn 5, 25 Stigmastadienol, and Momordicine I (right), and glycosides (above). (10)(11)

Article II Figures

Figure 1. Timeline of events for experimental procedures

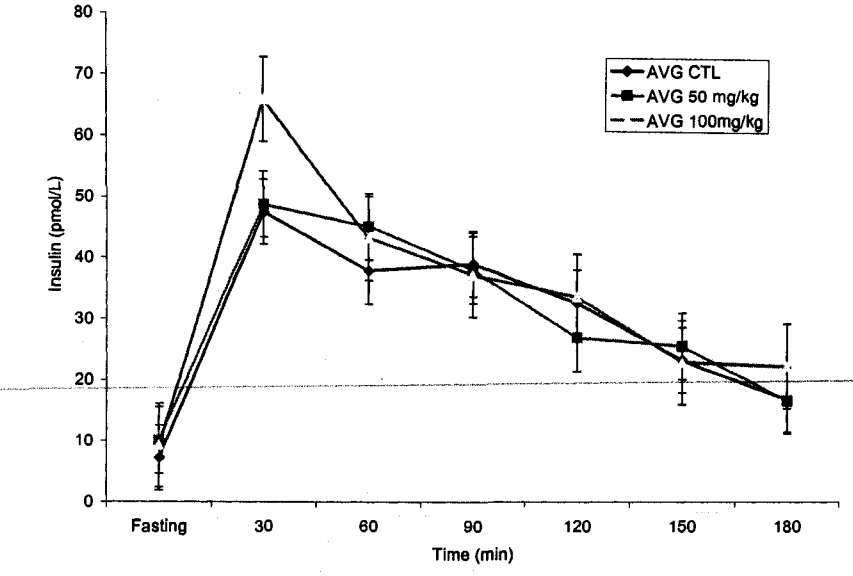


↑ Blood Sample

↑ Energy Expenditure Sample

↑ Visual Analogue Scale Questionaire (VAS)

Figure 1: A visual representation of experimental procedures during each session respectively.



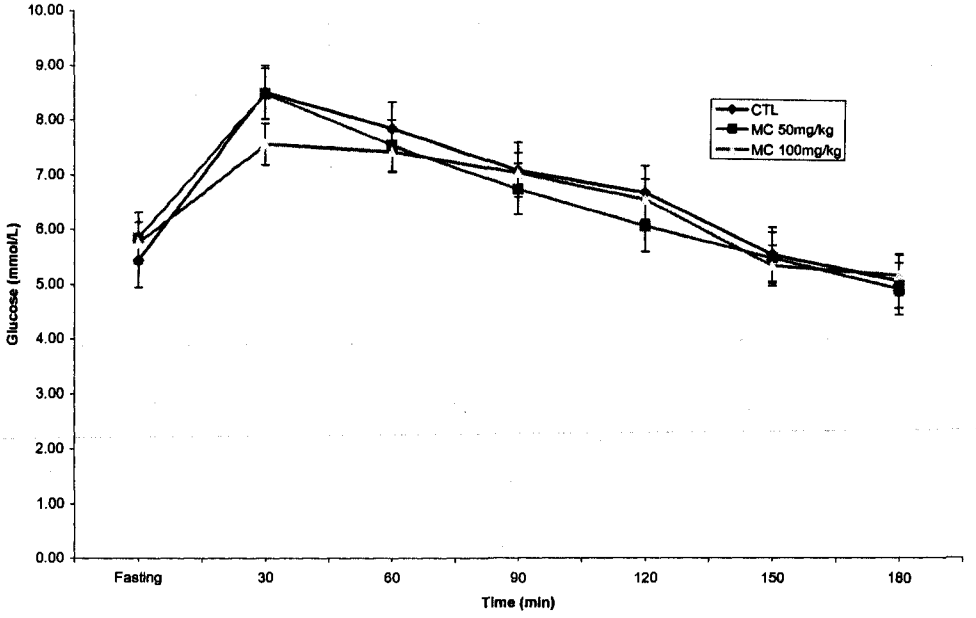
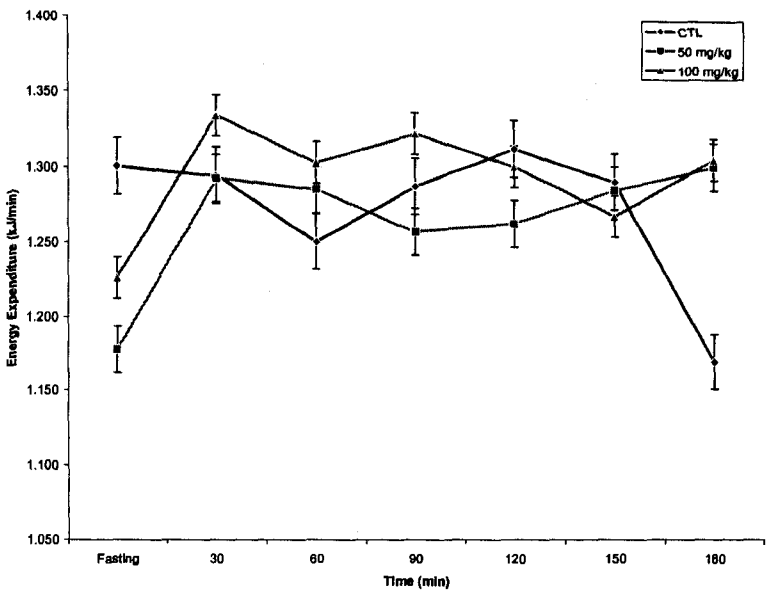
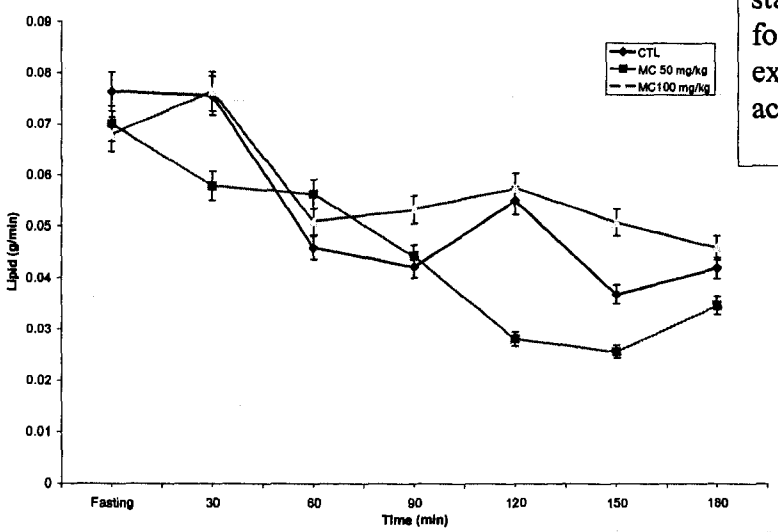


Figure 2 (Below). Plasma glucose (A) and (B) insulin concentration before and during an oral glucose tolerance test (75g) in healthy men supplemented with no MC (Ctl), 2) 50 mg/kg (MC50) or 3) 100 mg/kg of freeze dried MC (MC100). Values are mean ± SD. No statistical difference was found between

(A)



(B)



(C)

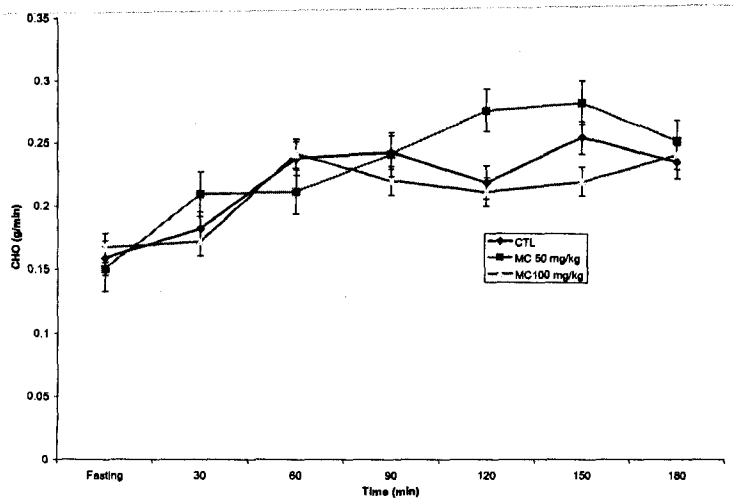
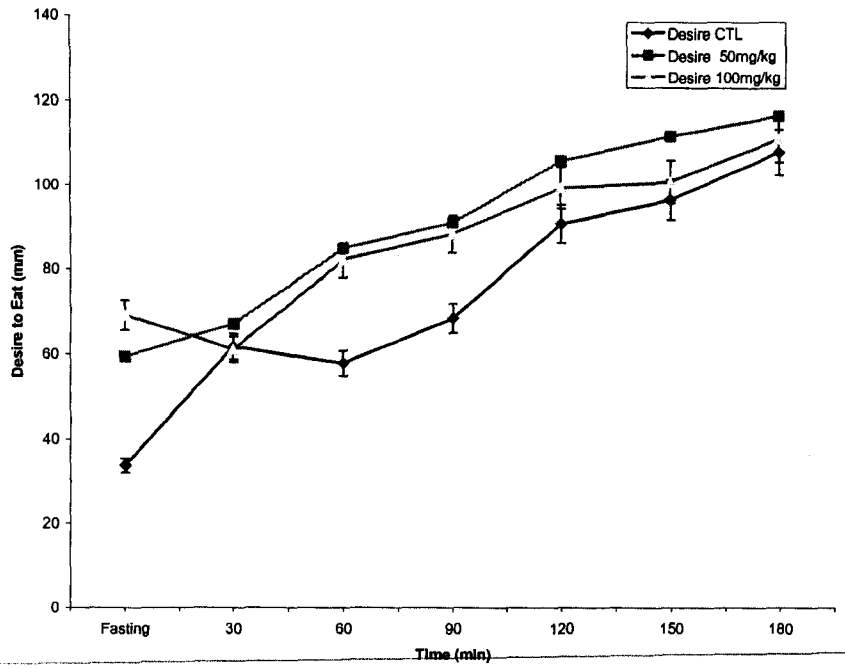


Figure 3 : (A) Resting Energy Expenditure (REE) kJ/min (SD+- 0.153kj/min) (B&C) Glucose and Lipid substrate oxidation (SD(+- 0.01g CHO, 0.01 Lipid) before and during an oral glucose tolerance test (75g) in healthy men supplemented with no MC (Ctl), 2) 50 mg/kg (MC50) or 3) 100 mg/kg of freeze dried MC (MC100). No statistical difference was found between experimental conditions across time ($P<0.05$)



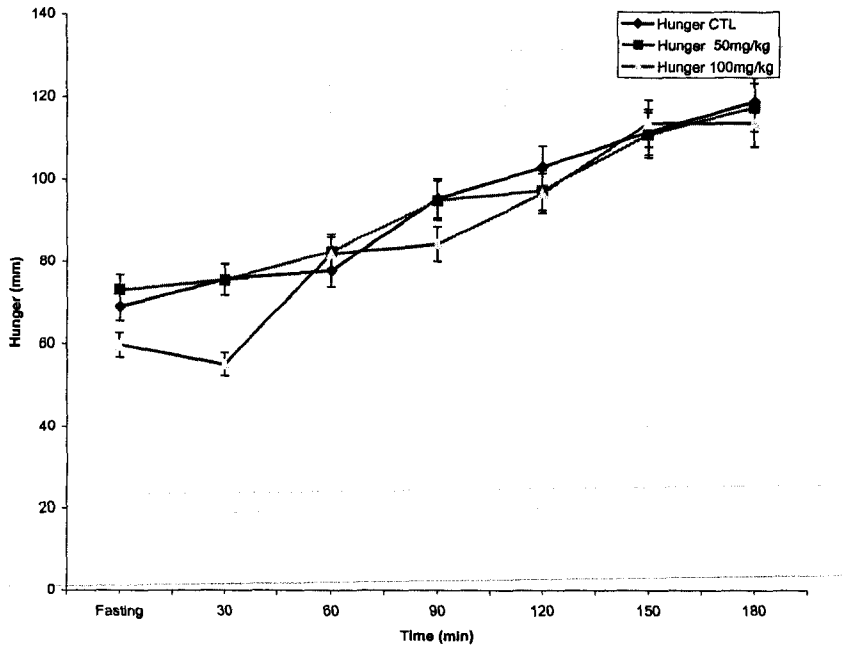
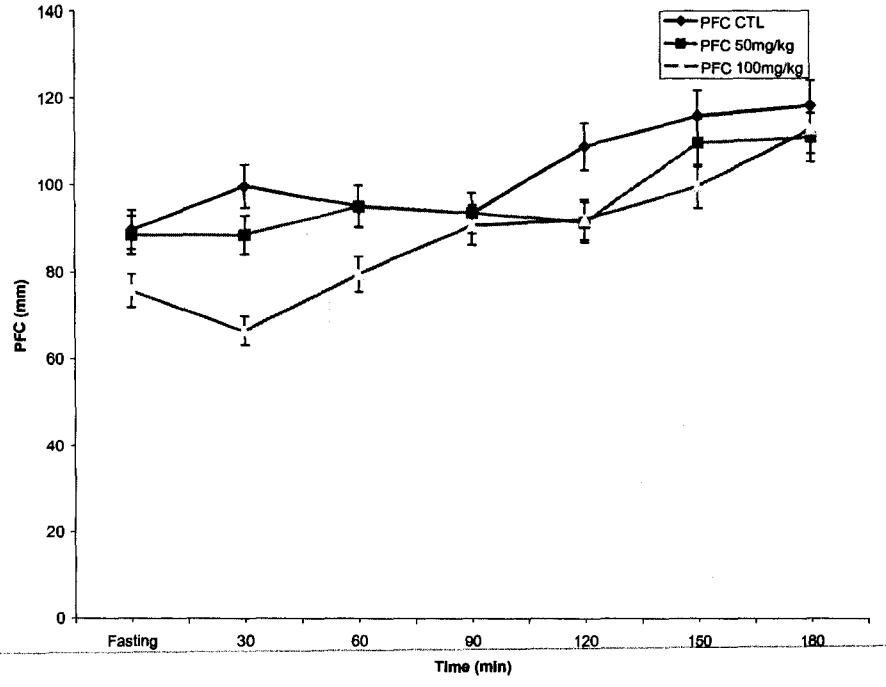
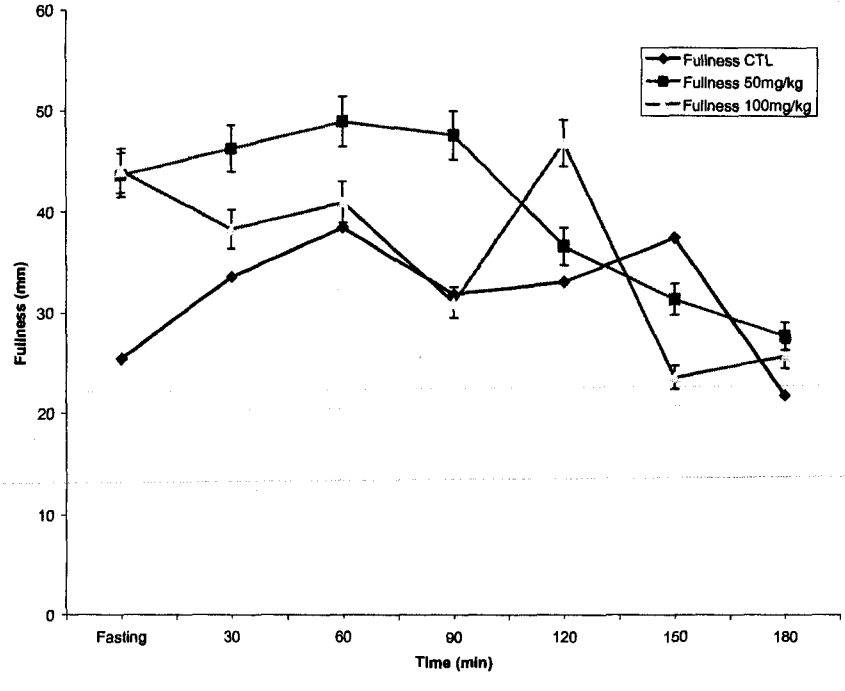


Figure 4 (below): Mean (SD) appetite scores before and during an oral glucose tolerance test (75g) in healthy men supplemented with no MC (Ctl), 2) 50 mg/kg (MC50) or 3) 100 mg/kg of freeze dried MC (MC100) for the desire to eat, hunger, fullness, and prospective food consumption (PFC) derived with the use of visual analogue scales throughout the control session (Control), low dose (50mg/kg) and high-dose (100mg/kg) testing sessions. (n=5, $p < 0.05$). No statistical difference was found between experimental conditions across time.


PFC CTL
PFC 50mg/kg
PFC 100mg/kg
PFC (mm)
140
120
100
80
60
40
20
0
Fasting
30
60
90
120
150
180
Time (min)


Fullness CTL
Fullness 50mg/kg
Fullness 100mg/kg
Fullness (mm)
60
50
40
30
20
10
0
Fasting
30
60
90
120
150
180
Time (min)

UNIVERSITY OF OTTAWA ETHICS APPLICATION

October 27, 2004

Dr. Pascal Imbeault
Human Kinetics
University of Ottawa
125 Université
(Room 350)
Ottawa, ON K1N 6N5

Mr. Gursevak Kasbia

RE: The Effects of Mormodica Charantia (Bitter Melon) on Glycemic Control and Energy Expenditure in Obese Men (file H 09-04-07)

Dear Researchers,

You will find enclosed the Health Sciences and Science REB ethical clearance for the abovementioned research study.

Please note that it is the responsibility of the Researchers to:

a) Inform the ethics office of any changes in the research project; and
b) Fill out an annual status report to be sent to the Protocol Officer for Ethics in Research. Such report can be found on the ethics web site at: http://www.uottawa.ca/services/research/rge/rebs/download/rapport_annuel_projets_anglais.doc

A copy of this approval will be sent to Research Services, if necessary.

If you have any questions, you may contact me at the number 562-5387.

Sincerely yours,

Rita D'Alessandro
Protocol Officer for Ethics in Research
For Dr. Daniel Lagarec, Chair of the Health Sciences and Science REB

HEALTH SCIENCES AND SCIENCE RESEARCH ETHICS BOARD

CERTIFICATE OF ETHICAL APPROVAL

This is to certify that the University of Ottawa Health Sciences and Science Research Ethics Board has examined the application for ethical approval for the research project entitled **The Effects of Mormodica Charantia (Bitter Melon) on Glycemic Control and Energy Expenditure in Obese Men (file H 09-04-07)** submitted by Gursevak Kasbia who is supervised by Dr. Pascal Imbeault, both of the School of Human Kinetics, Faculty of health Sciences. The Board found that this research project met appropriate ethical standards as outlined in the Tri-Council Policy Statement and in the Procedures of the University of Ottawa Research Ethics Boards, and accordingly gave it a Category 1a (approval). This certification is valid for one year from the date indicated below.

October 27, 2004

Rita D'Alessandro
Protocol Officer for Ethics in Research
For Dr. Daniel Lagarec, Chair of the
Health Sciences and Science REB

Date

ETHICS APPLICATION FORM

Submission for research project evaluation

Please answer all the questions. This application form must be used by all researchers conducting research projects involving, in their methodology, participation of human beings (questionnaires, individual interviews, focus groups, testing of equipment, physical endurance tests, etc.). For research based on secondary use of data or for course outlines, please use one of the other forms available on this Internet site. You will find an ethics application checklist at the end of this form.

1. *Type of Research:*

Student's research for Master's Thesis

Note: Students submitting their **masters or doctorate thesis** should append their **thesis committee=s approval.** The REB members will not evaluate thesis proposals before the thesis committee has evaluated the project.

Documentation provided with this application:

Please check the following attachments when applicable:

_X_ 13 copies of application form
_X_ 4 copies of research proposal
_X_ 13 copies of recruitment text to participants, parents, community representative
____ 4 copies of written permission from participating institution (if applicable)
_X_ 13 copies of consent form or information letter on Faculty letterhead
____ 4 copies of assent form for children (if applicable)
____ 4 copies of interview guide, questionnaire, or other instrument (if applicable)
____ 4 copies of debriefing form (if applicable)
_X_ 4 copies of the thesis committee's approval (if applicable)
_X_ Other (specify): phone questionnaire for inclusion in study; medical and nutritional history.

2. *Investigator(s):*
(**Note**: Students should give a permanent postal and email address for future correspondence.)

Name: Gursevak Kasbia

Department/School: Human Kinetics

Faculty: Health Sciences

Address: 36 Balding Cres

Phone:

E-mail:

Preferred language of correspondence: English

3. *Research supervisor (if any):*
(**Note**: Part-time professors should give a permanent postal and email address for future correspondence.)

Name: Pascal Imbeault

Phone: (613) 562-5800 x4269

Department/School: Human Kinetics

Fax: (613) 562-5149

Faculty: Health Sciences

E-mail: imbeault@uottawa.ca

4. *Research project:*
Title of the research project:

The effects of Momordica Charantia (Bitter Melon) on glycemic control and energy expenditure in obese men.

Funding source (if any): Consortium national de formation en santé (CNFS)

Expected Date of Termination: September 2005

Faculty of Health Sciences

Summary of the research protocol (not exceeding 250 words) ***(Submit also the research proposal which should not exceed five pages):***

The purpose of this research project is to examine the effects of the fruit Momordica Charantia (MC) (Also known as Bitter Melon) on glycemia, and energy regulation (age=18-40) overweight (BMI≥27) men. A total of three sessions will be conducted with a dosage of Momordica Charantia (MC) administered in a randomized order: placebo, MC 50 mg/kg and MC 100 mg /kg bodyweight (Please see diagram in the research proposal). This dosage corresponds

to the approximate amount extracted from the different volume of juice as administered in previous literature. Each of these conditions will be separated by a 2-week wash-out period to ensure that no residual effects from the previous experimental session are carried forward. Prior to each session, the participants will be asked not to consume any food (fasting) for 12 hours, after which, the participant will consume a standardized breakfast*(800kcal breakfast) (8:40am) which will be provided at the lab. This standardized breakfast will either contain the placebo dosage or one of the MC dosages respectively within the orange juice provided. A cellulose placebo will be used due to the fact that it has no known insulin activating properties. The subject will then be required to wait approximately 0.5 hours to ensure the absorption of the MC. Noteworthy is the fact that cellulose will also be included in the other experimental solutions. Blood samples will be collected in tubes via a venous catheter from an antecubial vein at – -30 (8:00am), 0, 30, 60, 90, 120 minutes. Plasma insulin, glucose and C-peptide concentrations will be determined respectively. Finally, energy expenditure will also be measured using indirect calorimetric methods. The research will be conducted at the Behavioral and Metabolic Research Unit located at the Montford Hospital, Ottawa.
*standardized breakfast details can be found in the proposal which is enclosed.

5. *Research participants*

Describe the research participants. Be as specific as possible by indicating the number of participants, their status, their age, their characteristics, etc.

At least twelve overweight/obese (body mass index = > 27 kg/m^2, waist girth > 100cm) (practice of less than 30 minutes of continuous exercise/week at moderate intensity) men aged between 18 and 40 years, will be recruited through the university population. They must be healthy, non-diabetic, and free of any illnesses or medications that might influence the outcome of the program. They must wear a short sleeve t-shirt, and a pair of track/jogging pants for each session. The clothing worn must be the same for each of the sessions.

If the research involves only women or man, or only francophones or anglophones, or any other particular group while discriminating against another, indicate why such discrimination is appropriate for the study.

As the proposed study is part of a Master's thesis, and must be duly completed within less than a year's time, the temporal sequencing for the testing of female participants would make an acceptable timeframe of completion exceedingly difficult to achieve. Because such time constraints are imposed by hormonal fluctuations during the menstrual cycle, and because of the cost related to sexual steroid measurement, men were chosen for this study in order to facilitate the acquisition of preliminary data. These data will then permit the elaboration of a design that will include both men and women. Such known differences in energy metabolism between men and women, as well as budgetary constraints regarding the number of subjects, also prevent inclusion of both sexes in order to have sufficient statistical power to detect differences.

6. *Recruitment of participants*

Indicate how the participants will be recruited for this research ***(Submit any poster or advertisement or the text of any method used to recruit research participants).***

Subjects will be recruited by means of poster ads, which will be placed on postage boards available throughout the University of Ottawa. Please see appended poster ads (Appendix I).

If inclusion or exclusion criteria are necessary for the research methodology, explain how the screening of potential participants will be carried out and how the excluded candidates will be notified.

Participants interested by the research will first contact the researcher by phone. During this first contact (*Initial contact visit*), a medical screening questionnaire (Appendix II) will be filled-out over the phone. If the potential participants meet the inclusion criteria at this point, they will then be invited to meet with the investigator for an initial contact visit. The participants who correspond to the inclusion criteria at this point and who accept to participate will then be asked to read the informed consent letter (Appendix III), and recruitment text respectively (Appendix IV). Consenting potential participants will then take part in the screening visit, where they will be weighed and measured in order to determine their eligibility for complete participation in the study. Potential participants who then meet the inclusion criteria for body mass index and waist girth will be able to participate in the entire study. If a potential candidate does not meet the required criteria at any stage of the screening process, the investigator will then notify him and give the specific reason(s) why he cannot take part in the study.

If there is a risk of negative reaction on the part of excluded candidates, or any other inconveniences for the excluded candidates, describe the nature of such risks or inconveniences and the measures taken to minimize these risks or inconveniences.

This researcher does not foresee any such reactions or risks.

7. Participation in the research

Outline what the participants will be required to do. Indicate the number and length of sessions that each participant will participate in ***(submit also any questionnaire or other material distributed or administered to the participants).*** *Do not submit mechanical apparatus. Where scientific instruments involving covert or physical contact are to be used, provide a description of the apparatus, its function and how it will be used (e.g. Electrodes, sensory devices, etc.).*

Aside from the *Initial contact* visit (1-2 hrs), participants will be required to take part in a *Screening visit*: to determining anthropometric measurements (weight, height, waist girth, and percent body free fatty acid). Participants will also be required to take part in four different experimental Sessions during the study (approximately 3 hrs each). For all four randomly assigned experimental sessions, participants will have arrive at the laboratory early in the morning (8h00) after a 12-hour overnight fast and after having abstained from any vigorous physical activities for at least 48 hours. After anthropometric measurements have taken place they will be asked to consume a standardized breakfast (8:40am). It is important to note that

qualified research and medical staff will be on hand for all measurements during all visits and experimental sessions. Details of the measurements to be taken are as follows.

Anthropometric measurements

Weight, height, and waist girth will be measured using a standard medical scale and measuring tape. Body composition (percent body free fatty acid and percent lean mass) will be measured by dual energy X-ray absorptiometry (DEXA) (GE Canada, Toronto ON). For this measurement, participants will be required to lie on a table while a computerized; low-intensity ($1/20^{th}$ of the radioactive rays received in an average 8 hour exposure period to the sun) X-ray scan of body free fatty acid and lean mass is performed. The individuals will have been advised not to have consumed any alcohol products prior to testing. As well, they will have to urinate 30 minutes prior to the test. This measurement takes on average 20 minutes, and proceeds while the participant is fully clothed.

Resting Energy Expenditure

This measurement will be taken during each of the trials, after the ingestion of the standardized breakfast with the dosage of placebo/MC, whereby the subject will be on a hospital bed, and have an indirect calorimeter placed over the face. The calorimeter infuses a steady flow of oxygen into the mask, while the byproducts of human breathing are then taken for analysis. This allows for the patient to be in a situation by which their safety is regulated and ensured. They will be monitored for the whole trial, by the research team.

Pre-experimental requirements

At the end of the *Screening visit*, participants will receive detailed instructions regarding pre-experimental dietary recommendations to which they must adhere. These recommendations will include a dietary regime incorporating 55% carbohydrates, 30% lipids, and 15% proteins. Participants will be required to follow this regime for 3 days before each experimental session (thus, followed 3 times in total).

For each experimental session, the participants will be provided with standardized breakfast (2 whole wheat slices of bread, 20g of Peanut Butter, 20g of Raspberry jam, 75g of Mild cheddar cheese, and 250ml of Orange juice) The RN (Registered nurse) who will perform the blood sampling portion will use the following: sterilized catheter, 5 ml test tubes laced with aprotinin/ EDTA solution, sterilized syringes and latex gloves.

In total six (5ml x 6 samples = 35ml) blood samples will be collected. The first sample will be collected 30 minutes prior to the experimental session (t = -30), followed by samples to occur immediately (t= 0) and 30, 60, 90 and 120 minutes after ingestion of standardized breakfast. The experimental session will span a period of approximately 3.0 hrs.

Collected blood samples shall then be subsequently, centrifuged (2500RPM for 10 minutes), and frozen (-80°C), within hours of being taken (2-3hours). The samples will then be analyzed on site at the medical laboratory located in the Behavioral Metabolic Research Unit (BMRU) at

the Montfort Hospital, Ottawa, Ontario, Canada for the determination of the following parameters: Insulin and C-peptide via the radioimmunoassay method. Likewise, glucose will be measured with the use of a Beckman Glucose Analyzer.

Concurrently at the same time that the blood samples are being taken, visual analogue scores shall be filled out by the participants to assess the following parameters: appetite visual analogue score (AVAS), hunger visual analogue score (HVAS), satiety visual analogue score (SVAS), and amount of food that could still be ingested visual analogue score (FVAS) at (t = 8:35, t =9:05, t = 9:35, t = 10:05, t = 10:35, t = 11:05).

Experimental sessions 1, 2, 3 and 4

Participants will be subjected to the following measurements during *these sessions respectively*

Time	Action
7:50am	**Arrival at laboratory**
8:00 am	**Dexa Scan Taken** **Health questionnaire given**
8:30 am	**Insertion of a catheter in the antecubital arm vein Initial Blood Sample (-30min)** **Initial Blood Sample Given (-30mins)** **Hunger and Visual Analogue Scale (8:35am)**
8:40 am	**Ingestion of Standardized Breakfast** **Participant will then be asked to lie down on medical bed**
9:00 am	**Measurement of resting energy expenditure and free fatty acid oxidation will be initiated * (20 minute sample every 30 minutes)** **Blood Sample (0mins)** **Visual Analogue Scale (9:05am)**
9:30 am	**Resting Energy expenditure sample** **Blood sampling (30mins)** **Visual Analogue Scale (9:35am)**
10:00 am	**Resting Energy Expenditure sample** **Blood sampling (60mins)** **Visual Analogue Scale (10:05am)**
10:30 am	**Resting Energy Expenditure Sample** **Blood Sampling (90mins)** **Visual Analogue Scale (10:30am)**
11:00 am	**Resting Energy Expenditure Sample** **Blood Sampling (120mins)** **Visual Analogue Scale (11:05am)**
11:30 am	**Resting Energy Expenditure Sample** **Blood Sampling (120mins)** **Visual Analogue Scale (11:05am)**

12:00 am	**Resting Energy Expenditure Sample** **Blood Sampling (120mins)** **Visual Analogue Scale (11:05am)**

Insulin, C-peptide and Glucose

Levels of Insulin, as well as C-Peptide and glucose levels will be determined from catheter-derived blood samples, which will all be drawn by a qualified nurse. Samples will be drawn from the antecubital vein of each participant's non-dominant arm. An external laboratory (Montfort Hospital, Ottawa, Canada) will analyze the samples for determination of each of these blood based constituents.

Appetite ratings

Appetite will be evaluated by means of visual analogue scales (VAS). This is done with pen and paper. The visual analogue scales will be administered after each blood sample. (Appendix V)

8. Free and informed consent

*Indicate how you will obtain the free and informed consent of the research participants and, where applicable, of authorized representatives of children or other participants who are not competent to give consent. (**Note**: Children or other participants not competent to give consent should still be given an opportunity to express their wish to participate or not. A separate consent form should be provided to these participants.)* ***Submit also the consent form(s) or information sheet to be given to the research participants.***

During the *Initial contact* visit, if a candidate meets the inclusion criteria, the investigator will explain the research protocol to the subject. This will be done in order to explain in detail the requirements of the protocol and to answer all questions from potential participants. If candidates are still interested in participating in the study, the investigator will then distribute the consent form to the participant, as well as explain any details and answer any questions regarding it. Finally, the investigator will suggest that the potential participant take the consent form home for closer reading before signing it and taking part in the *Screening session*.

*Indicate if information and consent documents will be translated for participants who may be more comfortable understanding one specific official language (or other language, if applicable). If translation is not provided, explain the reasons why. (**Note**: The principle of informed consent involves adaptation of language so as to be understood by participants. Even if the study is conducted in one language only, it is important, if possible, to translate consent documents.)*

The consent forms will be available in French and English to respect the policy of the right to communicate in both official languages in the department of Human Kinetics and the University of Ottawa. Furthermore, recruitment ads and screening questionnaires will be available in French and English in respect of said policy.

9. Proportionality of harms and benefits

Indicate if the methods used risk causing harms or inconveniences to the research participants. Describe the nature of such harms or the potential consequences on any legal, physical, psychological or social aspect associated with each procedure in the research or the methods used.

The methods used in this protocol do not pose a great risk to health. However, it is important to note that blood sampling can cause a small bruise (haematoma) at the location of the puncture that can remain for a few days. In addition, although the risk of infection or inflammation is small, it remains a possibility. It is also possible that some participants will feel faint during the insertion of the catheter or during blood sampling. However, it must be noted that these procedures will be performed by a qualified nurse, and that all possible precautions will be taken to ensure that the participants' health is of the utmost level of safety. Measurements of energy expenditure at rest as well as measures of bodyweight and composition do not represent a risk to health, although it is possible that participants experience some anxiety or discomfort while breathing in the ventilated hood. It is important to note that this apparatus assures an adequate supply of fresh air during the measurement, which considerably reduces the discomfort associated with it. Finally with respect to the actual administration of the chemotherapeutic (Momordica Charantia) agent, the subjects may feel slight discomfort while ingesting the pill. However, it will be done with the aid of water, and a suitable period of time will be given for them to relax before the trial commences.

Evaluate the level of physical or emotional harms or discomfort that the research could create for the research participants (None, low, moderate or high).

It is possible that some participants could feel some (low) degree of emotional distress regarding laboratory settings and blood sampling.

Indicate the measures you have taken to minimize such harms.

At least two people (researcher, nurse or research assistant) will be present during all measurements. A qualified nurse will work in a sterile fashion to avoid any complications related to blood sampling. Also during the resting energy expenditure measurements, a researcher and the nurse will be standing close to the subject to ensure that the subject is in the utmost condition of safety

Justify the potential harms by describing the anticipated benefits of the research (for general knowledge and for the research participants), and the way these benefits will be maximized.

The methods used in this protocol have been designed to minimize the risk to the participants. In addition, these methods are widely used and generally recognized to be very safe. The risks related
to the participation in this study are thus very small. On the other hand, the anticipated results could help us understand what effects momordica Charantia could have on glycemic regulation in humans. As well, it could also help to identify whether MC could be used to help obese individuals increase insulin sensitivity, which is a major complication in the etiology of diabetes

and obesity. Furthermore, this research could help to open the door to further research on energy regulation in humans, and may provide insight into whether momordica Charantia could be a suitable treatment for obesity. These results could then lead to the elaboration of an experimental design that is based on a combination of strategies aimed at helping these individuals to maximize free fatty acid loss and improve their insulin sensitivity and ultimately their health and quality of life. Furthermore, participation in this study allows the participants to receive specialized body composition at no cost, which are otherwise very costly and often inaccessible. These tests, along with the results of the study, will yield important information about participants' body composition and metabolic physiology, which in turn can be used to communicate practical suggestions for weight maintenance. It is thus the strong opinion of this researcher that the benefits related to this program substantially outweigh the potential harms that could be suffered by participants.

10. *Privacy of research participant*

Specify how you will ensure the anonymity of the research participants. Explain how the participants will be protected if you are not using pooled data. Where participants are interviewed, state whether the interviewees will be quoted, and if so, how their anonymity will be ensured. If anonymity is not to be guaranteed, explain why and mention it on the consent form or information sheet given to the participant

Precautionary measures will be taken to insure the privacy of all research participants. The name of participants will not appear on any report. A numerical code will be used to identify participants on all research documents. If any information related to this research is used in future analyses, only the code will appear on all research documents.

11. Confidentiality of data

*Specify who will have access to the data collected, where the data will be stored, how long the data will be conserved, and what particular measures will be taken to assure its confidentiality. (**Note**: Generally, data should be conserved for a period of 5 to 10 years after time of publication.)*

All material that could identify the participant to this study or any other material collected during the study will be kept under the strictest conditions and will not be made public, except in cases required by law. The data collected will be kept in a locked filing cabinet in a secured office space which only the supervisor and researcher have access to. All computer files will be protected by username and password therefore increasing their safety. The computer with the data on it is in the same room as the locked filing cabinet. Data will be kept for 5 years following the publication of results and will then be destroyed.

12. *Attestation*

I agree to abide by the ethical guidelines and procedures of the University of Ottawa Research Ethics Boards, of the *Tri-Council Policy Statement*, of my profession or discipline, as well as of the institution in which the research is undertaken. I am aware of my responsibility to be familiar with these standards. I further agree to notify the appropriate Research Ethics Board of any substantive change in the use of human participants in this research and to comply with requests made by such Board during the life of this research.

Signature of the Investigator: _____ Date: _____

Signature of the Supervisor: _____ Date: _____

Please Note: For the purposes of this master's project only Dr. Imbeault and his graduate student Gursevak Kasbia will have access to the data

APPENDICES

Université d'Ottawa · University of Ottawa

Faculté des sciences de la santé / École des sciences de l'activité physique — Faculty of Health Sciences / School of Human Kinetics

Looking for Volunteers for
A study on the effects of **Bitter Melon** on glycemic control

(At the School of Human Kinetics, University of Ottawa)
Our Inclusion Criteria is as follows:

- ✓ Men aged between 18 and 40
- ✓ No major health problems (ex. diabetes, thyroid disorder);
- ✓ Sedentary (i.e. less than 2 exercise sessions per week)
- ✓ Non-smokers
- ✓ Body mass index greater than or equal to 27 kg/m^2

If you feel you meet these criteria, let us know. You may contact us at [redacted]

Study on Bitter Melon | Study on Bitter Melon | Study on Bitter Melon | Study on Bitter Melon | Study on Bitter Melon | Study on Bitter Melon | Study on Bitter Melon

Faculté des sciences de la santé / École des sciences de l'activité physique — Faculty of Health Sciences / School of Human Kinetics

À la recherche de Participants
Pour une étude sur les effets du **melon amer** sur le contrôle de la glycémie (À l'École des sciences de l'activité physique de l'Université d'Ottawa)

Voici les critères de sélection:

- ☑ **Homme âgé entre 18 et 40 ans**

☑ **Pas de problèmes de santé tels que le diabète ou désordre thyroïdien**
☑ **Sédentaire (i.e. moins de 2 sessions d'exercice par semaine)**
☑ **Non-fumeurs**
☑ **Avoir un IMC plus grand ou égal à** 27 kg/m^2

Pour obtenir plus d'information, appeler Gursevak au [redacted]

APPENDIX

L'étude sur Melon Amer | L'étude sur Melon Amer | L'étude sur Melon Amer | L'étude sur Melon Amer | L'étude sur Melon Amer | L'étude sur Melon Amer | L'étude sur Melon Amer

Pre-screening questionnaire for the study entitled:

The effects of Momordica Charantia (Bitter Melon) on glycemic control and energy expenditure in obese men.

Researcher:
Gursevak S Kasbia
Supervisor:
Pascal Imbeault (Ph.D.)
Faculty of Health Sciences, University of Ottawa
School of Human Kinetics

1) What is your age? _____

2) What is your body weight? _____

3) What is your height? _____

4) Do you smoke? **Yes ☐ No ☐**

5) Have maintained a stable body weight (± 2 kg) over the last 6 months? **Yes ☐ No ☐**

6) Are you active? **Yes ☐ No**

☐

If yes, for how many minutes/week do you exercise? _____

7) Do you take medication? **Yes ☐ No**

☐

If so, please list them? _____

_____

_____

_____

_____

8) Do you have diabetes? Yes ☐ No ☐

9) Do you have any heart problems? Yes ☐ No ☐

10) Have you received a medical check-up in the last 12 months? Yes ☐ No ☐

11) Do you have high-blood pressure? Yes ☐ No ☐

12) Do you have asthma or any other respiratory problems? Yes ☐ No ☐

13) Has your doctor ever told you had thyroid gland abnormalities? Yes ☐ No ☐

14) Do you have any other health problems that were not mentioned in this questionnaire? Yes ☐ No ☐

15) If yes, which ones? _____

16) Do you follow a diet according Canada's Food Guide to Healthy Eating? Yes ☐ No ☐

17) Do you follow regular eating patterns (Breakfast, Lunch, and Dinner)? Yes ☐ No ☐

Université d'Ottawa · University of Ottawa

Faculté des sciences de la santé
École des sciences de l'activité physique

Faculty of Health Sciences
School of Human Kinetics

Questionnaire de recrutement

Les éffets du Momordica Charantia (Melon Amer) sur la glycémie et dépense énergique en des hommes avec obésité

Chercheur:
Gursevak S Kasbia
Superviseur :
Pascal Imbeault (Ph.D.)
Faculté des Sciences de la Santé, Université d'Ottawa
École des sciences de l'activité physique

1) Quel est votre âge? _____

4) Quel est votre poids? _____

5) Quelle est votre taille? _____

4) Fumez-vous? **Oui ☐ Non ☐**

5) Avez-vous maintenu votre poids (± 2 kg) pendant les derniers 6 mois? **Oui ☐ Non ☐**

6) Êtes-vous actif? **Oui ☐ Non ☐**

Si oui, à raison de combien de minutes/semaine d'exercice? _____

7) Prenez-vous des médicaments? **Oui ☐ Non ☐**

Si oui, précisez? _____

8) Avez-vous le diabète? **Oui ☐ Non ☐**

9) Avez-vous des problèmes cardiaques? **Oui ☐ Non ☐**

10) Avez-vous reçu un examen médical au cours des derniers 12 mois? **Oui ☐ Non ☐**

11) Souffrez-vous d'hypertension? **Oui ☐ Non ☐**

12) Souffrez-vous d'asthme ou d'autres problèmes respiratoires? **Oui ☐ Non ☐**

13) Est-ce que votre médecin vous a déjà mentionné que vous aviez des problèmes de la glande thyroïde? **Oui ☐ Non ☐**

14) Avez-vous d'autres problèmes de santé qui n'ont pas été relevé par cet questionnaire? **Oui ☐ Non ☐**

15) Si oui, précisez? _____

16) Suivez-vous un régime alimentaire recommandé par le Guide alimentaire canadien? **Oui ☐ Non ☐**

17) Est-ce que vos heures de repas sont régulières (Déjeuner, dîner, et souper) Oui ☐ Non ☐

APPENDIX 3

Université d'Ottawa · University of Ottawa

Faculté des sciences de la santé — Faculty of Health Sciences
École des sciences de l'activité physique — School of Human Kinetics

CONSENT FORM

The effects of Momordica Charantia (Bitter Melon) on glycemic control and energy expenditure in obese men.

Researcher:
Gursevak S Kasbia
Supervisor:
Pascal Imbeault (Ph.D.) [(613) 562-5800 x. 4269]
Faculty of Health Sciences, School of Human Kinetics
University of Ottawa, Ottawa, Ontario, Canada, K1N 6N5

I, _____, am invited to participate in the research conducted by Gursevak S Kasbia of the School of Human Kinetics at the University of Ottawa. The study is funded by the the CNFS (Consortium National de Formation en Santé). I understand that the purpose of this study is to investigate the effect of Momordica Charantia (Bitter Melon (MC) on glucose and energy regulation in the body. Literature suggests that this fruit may help those who suffer from obesity, by promoting energy regulation, and helping with the sensitivity of insulin in the body. I understand that the MC is a fruit of the Dominican Republic.

The researcher has assured me that the dose of MC has been designed with my safety in mind. However, if I am a diabetic or have any other health problems, such as heart problems or respiratory problems, for example, I willnot be able to participate in this study. I understand that this fruit has glucose lowering properties, and as such I cannot participate if I am a diabetic or am on medications to treat hypoglycemia. I understand that the main effects of taking this food substance acutely are that it lowers my blood glucose and potentially increases my energy expenditure.

Also, to qualify for this study, I must be between the ages of 18 and 40 years, with no major health problems, have a body mass index greater than or equal to 27kg/m^2 and I must not be very active (I must be exercising less than 2 times a week, 30 minutes or less each time). I must be a non-smoker, I must have maintained a stable body weight for the last 6 months, that I must have had a medical check-up in the past 12 months. Finally, I must follow a regular diet according to Health Canada's food Guide, and consume a balanced diet on a regular schedule (i.e. Breakfast, Lunch and Dinner).

My participation in this study will consist of one pre-screening measurement (approximately 90 minutes) and 3 experimental sessions (approximately 240 minutes each) during which many measurements will be taken.

The experimental sessions will be performed between 8:00am and 12h05am. A detailed description of these three visits is given in the table herein below. Testing will take place at the Behavioral and Metabolic Research Unit (BMRU) at the Montfort Hospital, Ottawa, Ontario, Canada.

My participating in this study will allow me to receive an evaluation of my body composition, and be provided compensation for my participation. Furthermore, it will allow the researcher to understand further how Bitter Melon may effect energy regulation, and glucose regulation in the body. This may in turn benefit society by providing an alternative food source for the treatment of obesity and its complications. Therefore, if I am interested in obtaining information about the components of this study and to determine whether I qualify for participation, I can reach Gursevak S Kasbia, the investigator in this study at (---) ---- or the supervisor of this study Dr. Pascal Imbeault at (613) 562-5800 ext. 4269.

Pre-screening measurements will be necessary to ensure that I meet the selection criteria of this study. During the pre-screening session, I, the study candidate, will be weighed, measured for height and waist circumference, and have my percent body free fatty acid determined. As well, I shall be required to complete a health questionnaire, where any positive relative risk factors detected will result in me being disqualified from this study. The researcher will also consult with me during this session to assess if I would have some allergies. Note: <u>It is essential that I have seen a doctor and had a medical check-up in the last year</u>.

If my characteristics correspond to all the necessary criteria and I accept to participate in this study, I will then be subjected to the following, which will be randomly selected by the researcher the day of testing.

Date (dd/mm/yyyy):_____ Participant's initials: _____

Session	What Procedures will be Performed	Time

Pre-Experimental Session (1 Session)	**1. I am required to wear shorts and a T-shirt** **2. I will not have exercised 24 hours prior to this session and I will not have consumed alcohol** **3. I will have a DEXA analysis as well as anthropometric measurements (Height, Weight and Body Free fatty acid) taken of myself.** **4. I will be required to fill in a Health Questionnaire SEE BELOW**	**2 hours (120 minutes)**
Experimental Sessions (3 Separate Sessions/ Days 2,3 and 4)	**1. I am required to wear shorts and a T-shirt (the same as during my Pre-Experimental Session)** **2. I will not have exercised 24 hours prior to this session.** **3. I am required not to have eaten in the past 12 hours** **4. I will have a DEXA analysis taken of myself** **5. I will be required to eat a Standardized Breakfast provided to me, and consume one of the dosages of Bitter Melon, or a Placebo.** **6. I will be required to provide samples of blood for analysis. (Every Half Hour)**	**3 Hours 5 minutes (245 minutes)**
	7. I will be required to fill in Hunger and Appetite scales (Visual Analogue Scales) (Every Half Hour) **8. I will be allowed to consume water during these sessions.**	
Wash-out Period	**1. I will be required to fill in a dietary log 3 days prior to each of my experimental sessions** **2. I will be required to wait 14 days between experimental sessions before I can return again.**	**14 days (2 weeks)**

DESCRIPTION OF MEASUREMENT

Pre-Screening Session *(Day 1)*

8:00 Pre-screening and anthropometrics measurements

- A Dual Energy X-Ray Absorptiometry device measurement, used to determine body composition and bone density, (a low radioactivity device amounting to 1/20 of the radioactive rays received over an 8-hour period, or 15-20 minutes, in the sunlight) will be performed to estimate my proportion of free fatty acid and muscle tissue. During this measurement, it is recommended that I have urinated 30 min prior to the test, consumed no alcohol 24 hours prior to the test, and not have exercised 24 hours prior to the test. I will also be measured for height, weight as well as waist and hip circumferences.

10:00 Estimated End of Prescreening Consultation

Experimental Sessions (Days 2, 3, and 4)

7:50am Arrival at Lab

For each experimental session I will arrive at 7:50am, and will have fasted for a period of 12 hours prior to the experiment. I will not have performed any vigorous physical activity, or have consumed alcohol within the last 24 hours, and will have provided the evaluator with a food diary journal, which lists what I have consumed in the past 3-days. I will be required to change into my shorts and t-shirt before I begin my experimental session at 8:00am. At approximately 8:30 am, a catheter will be inserted in my forearm and I will commence my experimental session (elaboration below).

8:30am Insertion of the catheter and resting measurements

I will be inserted with a catheter in the ante-cubical vein of my forearm followed my blood being taken, (catheter = a thin flexible tube which placed in a vein which allows the sampling of blood inserted by a trained nurse). Following the initial blood sampling (8:30 am), I will be asked to consume a standardized breakfast (8:40 am) given to me by the researcher. This will consist of 2 whole wheat slices of bread, 20g of Peanut Butter, 20g of Raspberry jam, 75g of Mild cheddar Cheese, and 250ml of Orange juice. While consuming my breakfast I will also be asked to consume either the bitter melon (Momordica Charantia) or placebo extract which will be mixed in water. The mixture will be prepared for me, depending on what session I participate in (i.e. placebo, 50 mg/kg or 100mg/kg bodyweight). I will then be asked to rest for a period of 15 minutes on a bed (From 8:45am-9:00am) until the next blood sample. After this point blood samples will be taken every 30 minutes (9:00am, 9:30am, 10:00am, 10:30am and 11:00am , 11:35am and 12:05pm). While the blood sampling is taking place, I will be asked to complete Visual Analogue Scale scores for appetite, satiety, hunger, and food that I have consumed and energy expenditure measurements will be taken.

8:45am *Blood Sample and Energy Expenditure measurements*

At 8:30am a blood sample will be taken, as well as energy expenditure samples using the metabolic carts provided. The metabolic carts consist of a ventilated fume hood placed over the participant, with a separate tube attaching them to the machine for sampling gas values produced. This fume hood is ventilated with fresh air, and a separate tube allows for expired air to enter the analysis machine. After this point this operation will be performed every 30 minutes, until sampling time is finished (11:00am).

12:00 pm Session End

The catheter will be removed and I will be able to change and leave the experiment, after filling in a last visual analogue scale.

BLOOD SAMPLE ELABORATION

- After I arrive, an initial sample of my blood (at) 8:30am (-30min) will be taken. This will be followed with other samples every 30 minutes (9:00am, 9:30am, 10:00am, 10:30, 11:00am,11:30am,12:00am) while I am at rest. In all, 8 blood samples of 5ml will be taken from my arm, which is equivalent to 40 ml of blood (5 ml x 8 samples) during each testing day (Days 2-4). The catheter will be removed immediately after I am done the testing (at about 12:05am).

FORESEEABLE RISKS

- The researcher has explained to me that the risks associated with this study are low. The DEXA device poses little risk. I also understand that blood sampling also poses small health risks. However, a small bruise could be apparent for a few days where the catheter is removed. Since the catheter will remain inserted for a few hours, I may feel some degree of discomfort. It was also explained to me that the risks of infection, or phlebitis (inflammation of a vein) and of vasovagal shock (loss of consciousness) are low under such conditions, but nonetheless remain a possibility. However, I understand that a qualified registered nurse will perform the insertion of the catheter and take the blood samples. I am aware that I am in close proximity to a Hospital (Montfort), in case of any emergency. The Bitter Melon (Momordica Charantia), is prepared in a food grade laboratory (Centre for Genomics Research, Arnason Laboratories). I will have access to drinking water, throughout the protocol, to consume at my discretion.

BENEFITS

- My participation in this study will allow me to gather information relative to my body composition and other health markers such as my waist circumference. It will also help the researchers and the scientific community to understand more about the role of Bitter Melon (Momordica Charantia) on energy and glucose regulation in the human body. Finally, a physical activity counseling visit will be offered during my participation in this study and afterwards, if I choose to receive consultation.

MONETARY COMPENSATION

- The researcher will compensate me by offering 50 dollars paid in increments at the beginning of each experimental session as follows: *($16.75/session)*. This amount compensates for my cooperation in this study and for parking and transportation costs. I will not receive compensation for any missed session(s).

CONFIDENTIALITY AND ANONYMITY

- I have received assurances from the researcher that the information I will share will be kept in the strictest confidentiality and anonymity.
    - My name will not appear on any reports and publications. A number code will be used to identify each participant, including myself, on all research documents.
    - All material and information that could be linked to me will not be made public and will be kept under the strictest confidentiality.
    - The data collected will be kept at the University of Ottawa in a locked filing cabinet in a room with limited access, meaning that the room can only be accessed by the researchers. The room is restricted, and has a pass code entry system to ensure only authorized personnel such as the researchers have access to it. In addition, the computerized data will be protected by a password. The data will be destroyed five years following its publication.

VOLUNTARY PARTICIPATION

- I am under no obligation to participate and if I choose to participate, I may withdraw from the study at any time. I may also refuse to answer any questions, without suffering any negative consequences. If I choose to withdraw, all data gathered until the time of withdrawal will nonetheless be used for the study.
- I will also be made aware of new findings that might influence my decision to take part in the present study.

RIGHTS OF THE PARTICIPANTS

The researcher assures me that:

- I can withdraw from the project at any time.
- The confidentiality of my information as well as my anonymity will be rigorously protected as indicated above.
- I have the right to be addressed in the language of my choice (English/French)

ACCEPTANCE

I,_____ , agree to participate in the above research study conducted by Gursevak S. Kasbia of the Faculty of Health Sciences, School of Human Kinetics, at the Behavioral and Metabolic Research Unit at the Montfort Hospital, Ottawa, Ontario, which is under the supervision of Dr. Pascal Imbeault. I understand that I am free to withdraw from the study at any time without suffering any consequences.

If I have any questions about the conduct of the research, I may contact the researcher or his supervisor at the following numbers: Dr. Pascal Imbeault at (613)562-5800 x. 4269, 350 Montpetit Hall, imbeault@uottawa.ca or Gursevak S Kasbia at :

If I have any ethical concerns with regards to my participation in this study, I may contact the Protocol Officer for Ethics in Research, University of Ottawa, Tabaret Hall, 550 Cumberland Street, room 159, Ottawa, ON K1N 6N5, tel.: 613-562-5841, email: ethics@uottawa.ca.

There are three copies of this consent form, one for the researcher, one for the supervisor, and one for me to keep.

Research Participant's signature: _____ Date: _____

Researcher's signature: _____ Date: _____

Supervisor's signature: _____ Date: _____

Université d'Ottawa · University of Ottawa

Faculté des sciences de la santé
École des sciences de l'activité physique

Faculty of Health Sciences
School of Human Kinetics

FORMULAIRE DE CONSENTEMENT

Les effets de Momordica Charantia (Melon Amer) sur la glycémie et la dépense énergétique chez des hommes caractérisés par une surcharge corporelle

Chercheur:
Gursevak S Kasbia
Superviseur:
Pascal Imbeault (Ph.D.) [(613) 562-5800 x. 4269]
Faculté des sciences de la santé, École des sciences de l'activité physique
Université d'Ottawa, Ottawa, Ontario, Canada, K1N 6N5

Je, _____, accepte de participer à cette recherche dirigée par Gursevak Kasbia de l'École des sciences de l'activité de l'Université d'Ottawa supportée par le Consortium National de la Formation en Santé. Je comprends que l'objectif de cette étude est d'examiner les effets de Momordica Charantia sur la glycémie (niveau de sucre dans le sang) et la dépense énergétique. La littérature indique que ce fruit peut avoir un effet positif pour les gens souffrant d'obésité en facilitant la dépense énergétique et en aidant la sensibilité de l'insuline dans le corps. Je suis conscient que le Momordica Charantia (melon amer) que je vais consommer est un produit de la République Dominicaine.

Je suis conscient que les problèmes associés à la dépense énergétique et à la régulation du glucose sont liés au taux croissant d'obésité en Amérique du Nord depuis les dernières décennies. Je comprends que les effets du fruit sur mon corps sont temporaires et pour la durée de l'expérience seulement. La quantité administrée a été décidée en prenant compte de ma sécurité. Je suis également conscient que si je suis diabétique, ou tout autres problèmes de santé, je **je ne pourrai pas participer à cette étude**. Je suis conscient que ce fruit peut abaisser le taux de glucose sanguin, donc je ne peux pas participer si je suis diabétique ou si je prends tout autres médicaments traitant l'hypoglycémie. Les effets principaux suite à la consommation de ce fruit sont l'abaissement de mon glucose sanguin ainsi qu'une augmentation de ma dépense énergétique.

Ma participation consistera essentiellement à réaliser une session de recrutement (durée approximative de 90 minutes) et trois séances expérimentales (approximativement 180 minutes chacune) au cours desquelles plusieurs mesures seront effectuées. Les séances expérimentales se dérouleront entre 8:00am à 11h05am. À noter, qu'une description détaillée de ces trois sessions est décrite ci-dessous. Cette recherche aura lieu à l'unité de recherche sur le comportement et le métabolisme de l'Hôpital Montfort, Ottawa, Ontario.

Nous cherchons des hommes âgés de 18 à 40 ans, sans problème de santé majeur **et ayant un IMC égal ou plus élevé que 27kg/m^2**. Nous demandons que vous ne soyez pas très actif, donc moins de 2 sessions d'exercice par semaine **de 30 minutes chacune**. Vous devez être non-fumeur, avoir gardé un poids stable au cours des 6 derniers mois, avoir eu un examen médical complet dans la dernière année, suivre un régime alimentaire recommandé par le guide alimentaire canadien, et avoir des heures de repas régulières.

En participant à cette recherche, je recevrai une évaluation de ma composition corporelle et une compensation monétaire. De plus, ma participation permettra aux chercheurs de mieux comprendre l'effet du melon amer sur la dépense énergétique et le taux de glucose sanguin. Ceci apporterait un traitement alternatif pour l'obésité et les problèmes reliés à ce dernier. Si je suis intéressé à recevoir plus d'informations au sujet de cette recherche et à savoir si je respecte les critères de sélection, je peux contacter Gursevak Kasbia, l'investigateur de cette recherche, au (---) ---, ou Pascal Imbeault, le superviseur de cette recherche, au (613) 562-5800 poste 4269.

Sessions de recrutement

- Je comprends que toutes les mesures effectuées au cours de la session présélection sont nécessaires afin de s'assurer que je satisfasse les critères de sélection de cette étude. Pendant cette séance, mon poids corporel, la circonférence de ma taille et ma composition corporelle (proportion de masse grasse et musculaire) **seront mesurés**.
- Je remplirai des formulaires relatifs à mon histoire médicale et nutritionnelle. Si certains risques reliés à la participation de cette étude sont détectés au cours de ces formulaires, je ne pourrai prendre part à l'étude. Cette session sera aussi l'occasion pour évaluer si j'ai des allergies et des aversions pour la nourriture que j'aurai à consommer au cours de l'expérimentation.

  Il est impératif que j'aie eu une évaluation médicale au cours des 12 mois avant cette recherche.

Sessions	Procedures	Temps
Pre-Experimental Session (1 Session)	**1. Je dois porter un chandail à manches courtes et un pantalon court** **2. Les mesures DEXA seront prises.** **3. Je devrai être à jeun 12 heures avant chaque session expérimentale alors que des échantillons sanguins seront effectués.** **4. Je dois completer une Questionnaire du Santé**	**2 Heures (120 minutes)**
Sessions Expérimentales Jours 2,3 et 4)	**1. Je dois porter un chandail à manches courtes et un pantalon court** **2. Les mesures DEXA seront prises.** **3. Je devrai être à jeun 12 heures avant chaque session expérimentale alors que des échantillons sanguins seront effectués.** **4. Des échelles visuelles analogues pour la mesure d'appétit seront admistrées** **5. Je ne devrai pas avoir fait d'exercice ou consommer de l'alcool dans les 24 heures avant chaque session.** **6. Je pourrai boire de l'eau en tout temps durant les sessions.**	**3 Heures 5 minutes (245 minutes)**

Period Libre	**1. Je devrai remplir un journal alimentaire 3 jours avant chaque session.** **2. Une fois l'une de ces conditions expérimentales complétées, une période d'au moins 14 jours séparera la prochaine session expérimentale**	**14 Jours (2 semaines)**

Date (dd/mm/yyyy):_____ Participant's initials:

_____

DESCRIPTION DES MESURES PRISENT

Session de recrutement

8h00 Mesures anthropométriques

- L'estimation de ma composition corporelle (i.e. proportion de matière grasse et de muscles) sera réalisée par un DEXA (Dual Energy X-Ray Absorptiometry). Le DEXA est un appareil très efficace utilisé pour déterminer le pourcentage de matière grasse et de muscles. Ceci n'implique aucun risque pour le participant sinon qu'une légère dose de radiation qui correspond à celle d'une exposition au soleil de 15-20 minutes. Au cours de cette mesure, je devrai être allé à la salle de bain au cours des 30 dernières minutes, ne pas avoir consommé d'alcool au cours des dernières 24 heures et ne pas avoir fait d'activité physique au cours des dernières 24 heures.
- Au cours de cette séance les mesures de ma taille, mon poids corporel et de ma circonférence de la taille seront effectuées.

10h00 Fin de la session de recrutement et des mesures anthropométriques

Jours 2, 3, et 4
7h50 Arrivée au laboratoire

Pour chaque session expérimentale, j'arriverai à 7:50am et je devrai ne pas avoir mangé au cours des derniers 12 heures. Je devrai avoir respecter de ne pas avoir fait d'exercice vigoureux ou consommé d'alcool dans les dernières 24 heures. Je devrai remettre mon journal alimentaire de 72 heures au chercheur. Aussi je dois me changer en t-shirt et short, pour être prêt à commencer la session expérimentale à 8 :00am.

8:30 L'insertion du cathéter
L'insertion d'un cathéter et les mesures de repos
- Un cathéter (i.e. un tube flexible et mince qui permet l'échantillonnage sanguin) sera inséré au niveau de la veine
antécubitale de mon avant-bras, suivi d'une prise sanguine à 8 :30am. Je serai ensuite invité à consommer un déjeuner standard (8:40am) composé de deux tranches de pain rôti, une cuillerée à table de beurre d'arachide, une cuillerée à table de confiture, une tasse de jus, et une portion de fromage. Au même moment, je vais boire la dose du Melon Amer (50mg/kg ou 100mg/kg du poids) (ou le placebo) dissout dans l'eau. Je devrai ensuite m'allonger sur un lit pour une période de 15 minutes (8 :45am à 9 :00am) jusqu'à la prochaine prise de sang. Mon métabolisme de repos sera aussi mesuré. Ceci sera fourni par les chercheurs. Après le déjeuner, une prise de sang

sera effectuée à toutes les 30 minutes pour une durée de 240 minutes. Suite à chaque prise de sang, je devrai remplir une échelle visuelle analogue pour la mesure d'appétit et mon métabolisme de repos sera aussi mesuré.

8 :45 *Échantillon de sang et mesures de dépenses énergétiques*

A 8:30, un échantillon de sang ainsi que les échantillons de dépenses énergétiques seront prises. Les mesures des dépenses énergétiques seront prises à l'aide de la carte métabolique. La tête du participant sera couverte d'une toile aérée. L'aire expirée est passe par un tube, et est ensuite analysée par la machine. Cette démarche sera répétée chaque 30 minutes, jusqu'à 12:00pm.

12:00 Fin de la séance

Le cathéter va être enlevé et je peux me rechanger dans mes vêtements et partir.

ÉLABORATION SUR LES ÉCHANTILLIONS SANGUINS PRÉLEVÉS

- Au total, 6 échantillons sanguins seront prélevés au cours de chaque session expérimentale (5 ml x 6 échantillon = 30 millilitres). À titre récapitulatif, à mon arrivée ~~au laboratoire, un premier échantillon sera prélevé à 8:30am (-30min). Un échantillon~~ sanguin sera prélevé immédiatement après le déjeuner ainsi que 30, 60, 90 et 120 minutes après ce dernier.
-

RISQUE(S) RELIÉ(S) À LA PARTICIPATION

Je comprends que les risques associés à cette approche sont très minimes. Je suis ou courant que les prises de sang sont associées à très peu de risque pour la santé, sinon que la possibilité d'apparition d'une ecchymose (un bleu) suite à l'installation du catéther. Par ailleurs, je sais qu'un certain inconfort peut être associé à l'insertion du cathéter. À l'extrême, certains risques tels une infection, une phlébite (Inflammation de la veine) ou un choc vasovagale (perte de connaissance) peuvent survenir lors de l'insertion du catéther, bien que cette probabilité demeure très minime. Cependant, je suis au courant que l'insertion de ce catéther et les échantillonnages sanguins est assurée par un/une infirmier(e) qualifié(e). Je comprends aussi que je suis à proximité de l'hôpital Montfort en cas d'urgence. Pour les autres mesures, il y a peu de risques associés à ces derniers. Le melon amer (Momordica Charentia) est préparé dans un laboratoire (Centre for Genomics Research, Arnason Laboratories). Je pourrai consommer de l'eau durant toute l'expérience.

AVANTAGES

Je pourrai obtenir de l'information concernant ma composition corporelle et autres indicateurs de la santé. Je bénéficierai également d'une consultation nutritionnel et d'activité physique durant, ou après la session si je le désire. J'aiderai également les chercheurs à comprendre d'avantage le rôle du melon amer sur la régulation corporelle de l'énergie et du glucose.

COMPENSATION MONETAIRE

Je recevrai un montant de 16.67$ après chaque séance expérimentale effectuée (total 3 x 16.67$ = 50$) (provenant des fonds de recherche du Consortium national pour la formation en santé). Ce montant est alloué pour souligner ma coopération dans cette étude et couvrir certains frais tel le coût de transport et stationnement. **Je ne recevrai pas de compensation pour toutes sessions manquées.**

CONFIDENTIALITÉ ET ANONYMAT

Il m'a été garanti que l'information personnelle partagée sera conservée sous le couvert de la confidentialité. L'information transmise sera seulement utilisée dans le cadre de la présente étude dont le but est d'examiner comment **le melon amer** influence la **glycémie et l'adiponectine dans le corps**. Mon anonymat et ma confidentialité seront conservés selon les façons suivantes :

1- Mon nom n'apparaîtra sur aucun document. Un code numérique sera utilisé pour m'identifier sur tous les documents.
2- Mes résultats utilisés pour les analyses statistiques seront identifiés par mon code de participant.
3- Tous matériels et informations qui peuvent m'identifier ne seront pas publiés et seront conservés sous le strict couvert de la confidentialité. Les donnés seront conservées dans un classeur barré situé dans une salle à accès limité **à l'université d'Ottawa, donc seulement les chercheurs liés à cette présente recherche y auront accès**. **De plus, tous documents informatiques seront protégés par un mot de passe, sur un ordinateur que est dans cette salle.** Finalement, les données de cette recherche seront conservées pendant une période de cinq ans, et seront ensuite détruites après cette période.

PARTICIPATION VOLONTAIRE

- Ma participation à cette recherche est complètement volontaire. À n'importe quel moment pendant l'étude, la priorité pour mes intérêts sera de mise. Je pourrai abandonner à n'importe quel moment si je le désire.
- Toutes les données recueillies à mon sujet avant ma décision d'abandonner cette étude **seront quand même utilisées pour cette étude.** (A voir CONFIDENTIALITÉ ET ANONYMAT)
- Je serai informé de nouvelles informations qui pourraient influencer ma décision à participer à cette étude.

DROITS DES PARTICIPANTS

Le chercheurs m'assurent que :

- Je peux annuler ma participation à cette étude en tout temps et ceci sans préjudice.
- La confidentialité de l'information recueillie à mon sujet et mon anonymat seront protégés comme indiqué ci-dessus.
- J'ai le droit de recevoir toute information dans la langue de mon choix (Anglais or Français).

APPROBATION

J'accepte de participer au projet initié par Gursevak S Kasbia de la Faculté des

Sciences de la Santé, École des sciences de l'activité physique, Ottawa, Ontario, sous la supervision des Dr. Pascal Imbeault. Je comprends aussi que je peux annulé ma participation à l'étude en tout temps et ceci sans préjudice. Advenant le fait que j'aie des questions relatives à l'étude, je peux contacter le chercheur de cette étude ou l'un de son superviseur : Dr. Pascal Imbeault au (613)562-5800 x. 4269, pièce 350 Pavillon Montpetit, imbeault@uottawa.ca

Toute information concernant tes droits comme participant dans une recherche peut être adressée à l'agent de protocole pour éthique de recherche, 550, Cumberland St., room 159, (613) 562-5841 or ethics@uottawa.ca.

Il y a 3 copies de ce formulaire, une pour le chercheur, une pour les superviseurs, et l'autre m'appartient.

Signature du Participant: _____ Date: _____
Signature du Chercheur _____ Date: _____
Signature du Superviseur: _____ Date: _____

APPENDIX IV

Université d'Ottawa · University of Ottawa

Faculté des sciences de la santé / École des sciences de l'activité physique
Faculty of Health Sciences / School of Human Kinetics

Recruitment Text

The effects of Momordica Charantia (Bitter Melon) on glycemic control and energy expenditure in obese men.

Researcher:
Gursevak S Kasbia
Supervisors :
Pascal Imbeault (Ph.D.)
Faculty of Health Sciences, University of Ottawa
School of Human Kinetics

Hello,

My name is (researcher's name) and I am involved in a study on the effects of Momordica Charantia (Bitter Melon) on energy expenditure and glycemia (glucose utilization) that may further allow us to understand obesity. This study is done with the approval of the Faculty of Health Sciences, School of Human Kinetics at the University of Ottawa, under the supervision of Dr. Pascal Imbeault

We are looking for male participants between the ages of 18 and 40, with no major health problems. We also ask that you are not very active, in other words, exercise less than 2 times a week for bouts of 30 minutes or less. We also ask that you are a non-smoker, that your body weight has been stable within the last 6 months and that you have had a medical check-up in the past 12 months. We also ask that you follow a regular diet according to Health Canada's food Guide, and consume a balanced diet on a regular schedule (i.e. Breakfast, Lunch and Dinner).

By participating in this study, you will be given the opportunity to receive an evaluation of your body composition, and be provided compensation for your participation. Therefore, if you are interested in obtaining information about the components of this study and to determine whether you qualify for participation, you can reach Gursevak Kasbia, the investigator in this study at or the supervisor of this study Dr. Pascal Imbeault at (613) 562-5800 ext. 4269.

Université d'Ottawa · University of Ottawa

Faculté des sciences de la santé
Faculté des sciences de l'activité physique

Faculty of Health Sciences
School of Human Kinetics

Texte de Recrutement

Les effets du Melon Amer sur la glycémie et la dépense énergétique chez des hommes caractérisés par une surcharge corporelle

Chercheur:
Gursevak S Kasbia
Superviseurs :
Pascal Imbeault (Ph.D.)
Faculté de les Sciences de la Santé, Université d'Ottawa
École d'Activité Physique

Bonjour,

Je m'appelle (Nom du chercheur(e)) et suis impliqué dans une étude qui tentera d'examiner les **effets** de Melon Amer sur la glycémie et la dépense énergique en l'obésité. Cette étude est réalisée à l'École des sciences de l'activité physique, Faculté des sciences de la santé, Université d'Ottawa, sous la **supervision** de Dr. Pascal Imbeault.

Nous sommes à la recherche de participants masculins, âgés entres 18 et 40 ans, sans problème de santé majeure, pas actifs (i.e. ne pratique pas de l'exercice plus que 2 fois/semaine pour une période de 30 minutes par session) et non-fumeurs. Les participants doivent avoir passé un examen médical au cours des derniers 12 mois, avoir un poids stable depuis les derniers 6 mois, suivre une diète selon le Guide alimentaire canadien et avoir un horaire de repas régulier (Déjeuner, Dîner, et Souper).

En participant à cette étude, vous obtiendrez une évaluation de votre composition corporelle, et une compensation financière. Pour obtenir de plus amples informations à propos de cette étude, veuillez contacter Gursevak Kasbia, chercheur responsable de l'étude, au
ou **Dr.** Pascal Imbeault, superviseur de cette étude, au (613)562-5800 **poste** 4269.

APPENDIX

VISUAL ANALOGUE SCALE

PROJECT CIMR

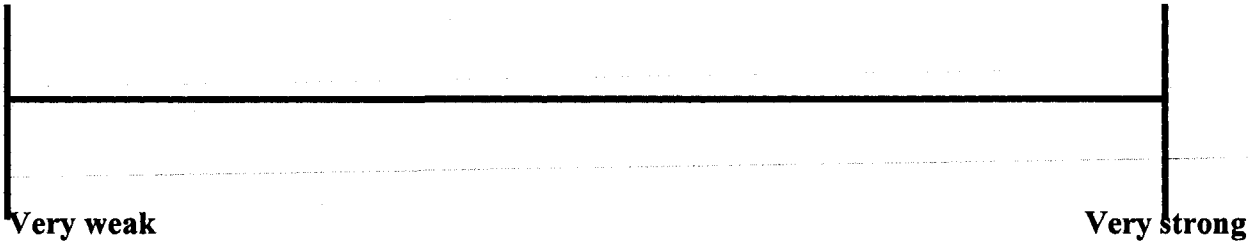
Visit	Date (dd/mm/yyyy)			Initials/subject			Subject Code			
RA							CIMR			

TIME: Pre-

Visual Analogue Scale (150 mm)

Procedure	Please quantify your sensation for the feeling mentioned below. Consider the line as two extremes of this sensation. Draw a vertical line that best represents this sensation at this moment in time.

1. **How strong is your desire to eat?**



PROJECT CIMR

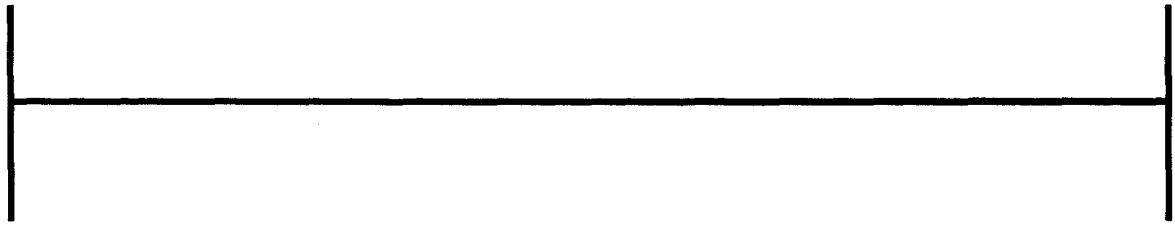
Visit	Date (dd/mm/yyyy)			Initials/subject			Subject Code			
RA							CIMR			

TIME: Pre-

Visual Analogue Scale (150 mm)

Procedure	Please quantify your sensation for the feeling mentioned below. Consider the line as two extremes of this sensation. Draw a vertical line that best represents this sensation at this moment in time.

2. How hungry do you feel?



Not hungry **at all**

As hungry as I **have ever felt**

PROJECT CIMR

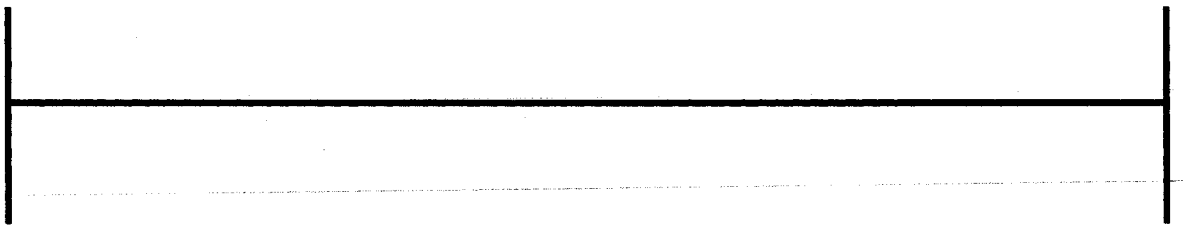
Visit	Date (dd/mm/yyyy)			Initials/subject			Subject Code			
RA							CIMR			

TIME: Pre-

Visual Analogue Scale (150 mm)

Procedure	Please quantify your sensation for the feeling mentioned below. Consider the line as two extremes of this sensation. Draw a vertical line that best represents this sensation at this moment in time.

3. How full do you feel?



Not full **at all**

Very full

PROJECT CIMR

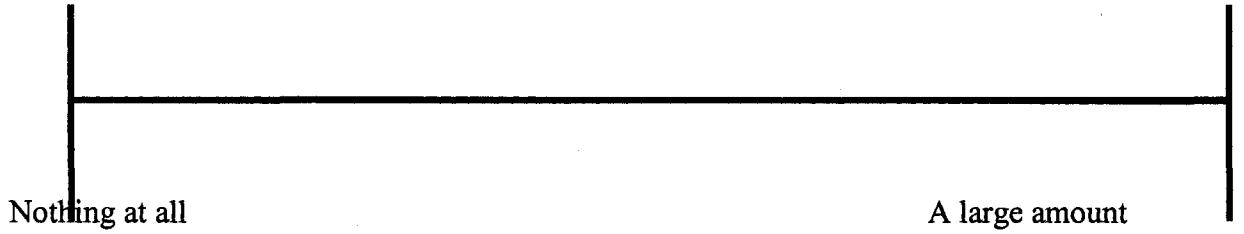
Visit	Date (dd/mm/yyyy)			Initials/subject			Subject Code			
RA							CIMR			

TIME: Pre-

Visual Analogue Scale (150 mm)

Procedure	Please quantify your sensation for the feeling mentioned below. Consider the line as two extremes of this sensation. Draw a vertical line that best represents this sensation at this moment in time.

4. How much food do you think you could eat?



PROJET CIMR

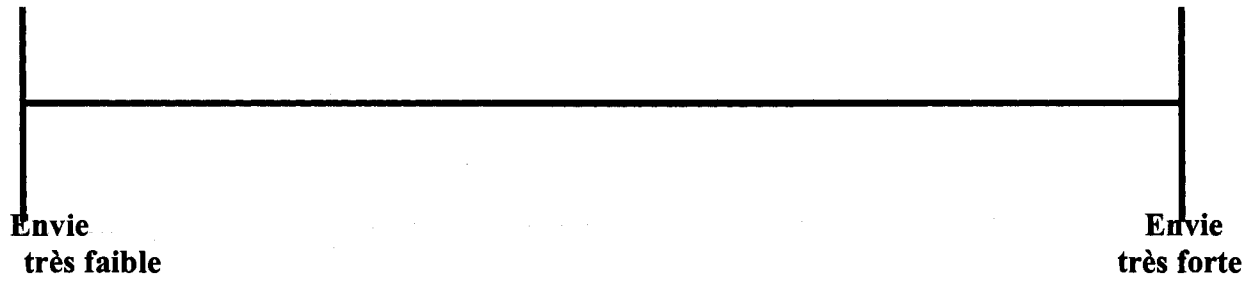
Visite	Date (jj/mm/aaaa)			Initiales/sujet			Code sujet			
TEMPS : Avant										

WHY SO MANY FRENCH VERSIONS?????

Échelle Visuelle Analogue (150 mm)

Consigne	**S.V.P. quantifiez votre sensation pour l'aspect mentionné ci-dessous. Considérez la ligne comme étant les écarts extrêmes de votre sensation. Tracez un trait vertical sur cette ligne au niveau qui représente le mieux votre sensation à ce moment précis.**

5. Dans quelle mesure avez-vous envie de manger?



PROJET CIMR

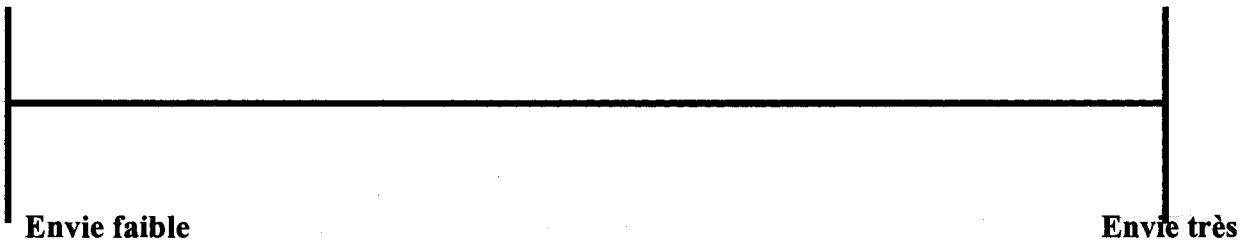
Visite	Date (jj/mm/aaaa)			Initiales/sujet			Code sujet			

TEMPS : Avant

Échelle Visuelle Analogue (150 mm)

Consigne	**S.V.P. quantifiez votre sensation pour l'aspect mentionné ci-dessous. Considérez la ligne comme étant les écarts extrêmes de votre sensation. Tracez un trait vertical sur cette ligne au niveau qui représente le mieux votre sensation à ce moment précis.**

6. Dans quelle mesure avez-vous l'impression d'avoir faim?



Envie faible

Envie très très forte

PROJET CIMR

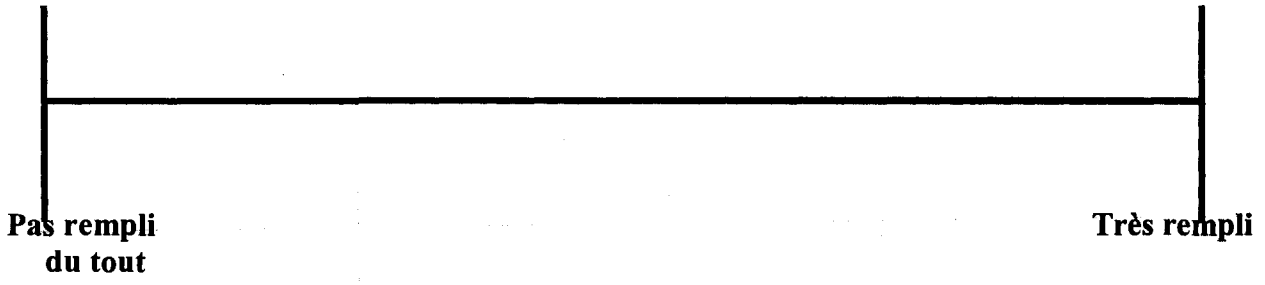
Visite	Date (jj/mm/aaaa)			Initiales/sujet			Code sujet			

TEMPS : Avant

Échelle Visuelle Analogue (150 mm)

Consigne	**S.V.P. quantifiez votre sensation pour l'aspect mentionné ci-dessous. Considérez la ligne comme étant les écarts extrêmes de votre sensation. Tracez un trait vertical sur cette ligne au niveau qui représente le mieux votre sensation à ce moment précis.**

7. À quel point vous sentez-vous rempli?



PROJET CIMR

Visite	Date (jj/mm/aaaa)			Initiales/sujet			Code sujet			

TEMPS : Avant

Échelle Visuelle Analogue (150 mm)

Consigne	**S.V.P. quantifiez votre sensation pour l'aspect mentionné ci-dessous. Considérez la ligne comme étant les écarts extrêmes de votre sensation. Tracez un trait vertical sur cette ligne au niveau qui représente le mieux votre sensation à ce moment précis.**

8. Quelle quantité de nourriture pourriez-vous manger immédiatement?

Absolument **rien**

Une grande **quantité**

Thesis Proposal: School of Human Kinetics.

Momordica Charantia: A bitter sweet potential for the treatment of Insulin Resistance

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Background: The objective of this study is to determine the effect of Momordica Charantia (MC) on glycemia in obese individuals. This will be accomplished by supplementing MC, in a randomized placebo based design. A total of 24 participants will be recruited who are considered clinically obese (BMI $\geq$27), aged between 18 to 40 years of age. Specifically, the main objective of this research is to determine the effect of MC on fasting and post-prandial glucose homeostasis, as well as a potential link between MC and resting energy expenditure. Research has primarily been done on diabetic induced allcoxan, KK-Ay and STZ rats and has been limited in human models. Thus far a positive association has been observed between the consumption of MC, and a hypoglaecemic effect, as seen in the diabetic animal models previously mentioned. As for energy expenditure, limited study has been done in this area and it is thus within the researchers interest to observe whether MC will in fact have an effect on resting energy expenditure. In terms of a treatment for the epidemic of obesity, this could benefit those who are suffering from such conditions as for impaired glucose tolerance. As well, if energy expenditure is seen to increase, this would have a beneficial impact on those who battle obesity daily. Since obesity and diabetes have a close relationship it is thus very important for research in this area to explore the impact that this nutritional product will have on glucose homeostasis and energy expenditure, with respect to the growing problem of the metabolic syndrome.

Keywords: hypoglycaemia, Momordica Charantia, impaired glucose tolerance, obesity, metabolic syndrome, Human.

Introduction:

Obesity and its complications are a growing worldwide trend that has many costly implications(2). Obesity has been associated with many of the world's sedentary diseases, namely diabetes and cardiovascular disease(3, 4). Adiposity has been reported to have a potent affect on insulin resistance. Insulin resistance has been defined as the reduced biological action of insulin. Insulin sensitivity decreases in many cases of diabetes mellitus, and thus can have

serious repercussions for patients which will be explored further in this paper. Long term exposure to insulin resistance, impaires glycemic regulation in the body, which may then lead to complications associated with diabetes. These include conditions such as retinopathy, neuropathy, and other degenerative physiological states(5). Nutritional intervention has been shown to delay the onset of non-insulin dependent diabetes mellitus(NIDDM), and complications associated with it (3, 6, 7). Hyperinsulinemia, and the resulting insulin resistance, have been known to be markers for the initial stages of diabetes(4). Prevention by means of dietary intervention and exercise offer a solution to this growing problem.

One plant that may hold the key to reducing the complications of diabetes is referred to as bitter melon. The scientific name of this plant is Momordica Charantia (MC), and is found typically in tropical type climates. Countries such as Thailand, India and Jamaica, have native species of these plants. It is a native plant species of the Cucurbitaceous family and has many medicinal properties associated with it. In the case of diabetes, it has been known to illicit a substantial insulin secretagogue effect. Since diabetic patients suffer from insulin resistance, momordica would act as an insulin secretagogue, and furthermore possesses properties to protect any remaining functional islet cells. This is important to those who suffer impaired glucose tolerance due to the fact that MC may then act to increase insulin sensitivity. Glucose may then be metabolized more efficiently, which occurs when NIDDM patients are administered oral hypoglycemics. Asides from the glucose lowering effect, momordica has also been seen to reduce hepatic glucose production(8), by reducing the amount of gluconeogenesis that normally occurs in diabetics. It accomplishes this by stimulating and modifying key enzymes used to mobilize glucose utilization hepatically, such as gluthionine S-transferase(9) and its precursor Gamma-glutamyl transpeptidase, and alkaline phosphatase(10). Finally in such conditions as diabetic neuropathy and retinopathy, momordica has been found to be very effective in their prevention by promoting the protection of pancreatic beta cells(1). This may in turn aid glucose homeostasis, and inhibit pathologies associated with diabetes and the metabolic syndrome(11, 12). Chronically speaking this may help those who suffer from these types of pathologies.

Mechanism of Action:

Insulin Resistance: A brief overview

Obesity has been associated with insulin resistance, which is one of the main factors that contribute to the etiology of NIDDM. There are a multitude of different mechanisms surrounding insulin resistance, which include the impingement in adipose tissue blood flow(13), and or decrease in vascular action of insulin(14). The modern calorically dense diet of North Americans may be responsible for problems with respect to insulin sensitivity. Hyperinsulinemia has also been implicated as a major determinant per se to insulin resistance. Since many individuals react differently to glucose metabolism, and homeostasis, the onset of hyperinsulinemia may differ in individuals. However, hyperinsulinemia ultimately will lead to insulin resistance, and ultimately Beta cell failure(15). This is severely problematic due to the fact that insulin is the main factor that allows the human body to store glucose, and maintain glucose homeostasis. Receptors for insulin are also a main culprit of this syndrome, due to the fact that although there may be enough receptors, they may be malfuntional. This can interrupt the flow of glucose into the cell, and may resultantly cause a greater increase in insulin in the blood. This complicated physiological problem of obese patients suffering diabetes has commonly been referred to as the metabolic syndrome X. As well, specific adipocytokines, which have been linked with pro-inflammatory type actions, have also been linked to the development of NIDDM(16). This may be due to the fact that certain adipocytokines such as tumour necrosis factor alpha (TNfα) may be linked with the release of excessive amounts of this specific immune agent. This may be a danger due to the fact that TNfα in particular is naturally occurring immune agent involved in cell apoptosis. However, in this case the healthy beta cells of the pancreas are instead targeted and thus leading to the ensuing NIDDM.

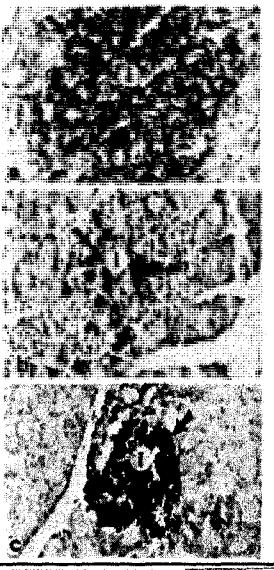


Figure 1: Visual representation of islet cells
(a) a normal rat islet cell (b) a diabetic rat islet cell (c) a diabetic rat islet after treatment with MC. Morphological change occurred following MC supplementation in alloxan rats.(1)

As an insulin secretagogue, MC has many active ingredients that have been isolated and acknowledged within the scientific community. These active ingredients include Charantin, Momordin I and II and 5, 25 stigmastendiol. These components have been linked to having phytosterol properties, and are also ribosomal inhibiting proteins (RIP)(17). This is of particular importance due to the fact that it has been shown that momordica juice extract, when given to STZ- induced diabetic mice, and allcoxan rats, allowed for the protection of islet cells(1, 18)(refer to Figure 1), which are also linked to the same cells which undergo irregular apoptosis in NIDDM patients. In the case of the mice that are used in these studies, STZ would refer to the usage of streptozotocin to wipe out the existing beta cell population(19). The similar situation occurs with KK-ay mice, which actually have the gene that is specific to the production of islet cells destroyed ("knocked out"), so that experiments may be performed to allow for a greater understanding of the etiology of insulin resistance and other pathologies and specific interventions/treatments.

Charantin is a 9.7 kDa napin like RIP, which has been extracted from the plant of MC(17, 20). Previous studies also extracted from the seed, however the seeds have been known to induce abortion type properties in females(21). For this reason females will not be included in the study at hand, for reasons of safety. Sterol based components may act synergistically with Charantin to produce the hypoglycaemic affect. For example, 5,25 stigmastendiol was isolated from the extraction of the fruit of Thai bitter melon. Both Momordin I and 2, 5 stigmastendiol were found to be the active components in the juice. When given to STZ induced diabetic mice, these components were found to have a significant effect in the lowering of plasma glucose(22).

How these phytochemicals may allow for the protection of cells, and stimulate the uptake of glucose may lie in thier impact on key enzymes in the body. For example tumour necrosis factor alpha (TNf α) has been associated with being a major determinant of inflammation, and has also been linked to being responsible for islet cell death(18, 23). Although the physiology of this mechanism has not yet fully been understood, it has been noted in the literature that the presence of high levels of TNf α have strongly been associated with insulin resistance. Hepatic uptake of glucose is also important, because this physiologically serves as a means for the body to be able to store glucose, and inhibit FFA synthesis(24). Based on animal studies, MC has also been

associated with hepatic glucose uptake(8, 10, 25). It may do this by stimulating key hepatic enzymes such as gluthionine S-transferase (GST) to function normally(9). This specific enzyme has been identified with protecting cells from oxidative stress, toxins and metabolites(26). As well, Shabib et al, has found that MC contributed to the depression of gluconeogenic enzymes glucose 6 phosphatase and fructose 1-6-biphosphate and elevated the liver enzyme glucose-6-phosphate dehydrogenase(8). This specific enzyme is very important in glycemic regulation in humans, because it allows for the breakdown of key glycogen stores and increased glucose utilization as a metabolic fuel, which may be hindered in individuals with insulin resistance.

Along with key enzyme regulation, the muscular concentrations for the protein based glucose transporter (GLUT-4) have also been reported to increase in animals. In a recent clinical study, allcoxan rats were given a treatment with MC juice(27). This was accompanied by an increase in the number of GLUT-4 receptors on the cellular surface. This particular protein is responsible for the facilitation of glucose transport into the cell due to the fact that it is receptor mediated via insulin.

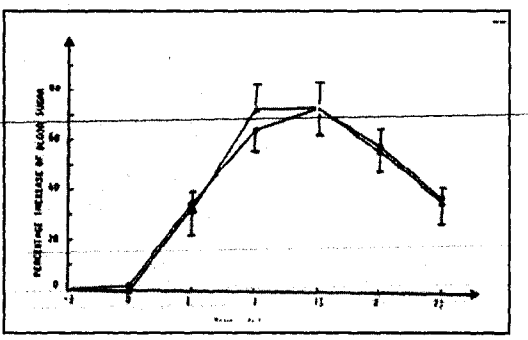
As far as the utilization of specific energy sources in the body, it was found in a recent study, that MC when supplemented in allcoxan rats increased the energy expenditure of rats versus that of the corresponding placebo group(28). Also, body composition changed, and less visceral adipose tissue was seen to deposit, thus revealing that there is a link between MC consumption and the potential to inhibit the onset of obesity. Another recent study also implicated exercise combined with MC consumption as having a significant effect on diabetic allcoxan rats. This trial also was much longer than those previously performed, and occurred under strict laboratory setting, with a high level of statistical power(29) (refer to Figure 2).

Dosage

Rat Studies

In recent studies, MC has been noted to be effective in STZ and allcoxan rats, however dosages have differed in range, and there have been a limited amount of human studies, that have critically taken into account this factor when MC has been given orally. In one study 500mg/kg prepared in from an alcohol extract were administered to STZ rats. It was observed that a ten to fifteen percent decrease in plasma glucose occurred. Within the third hour of the same study MC

elicited approximately 26-30 percent of what metformin® would normally depress glucose by. However, that being said, insulin levels were not increased, as per normal response which the authors hypothesized that the mechanism which may underline the action of MC may lie in the uptake of glucose hepatically (25). Virdi et al. administered three different dosages of MC respectively (20mg/kg at 5.6%, 4.8%, 4.1% concentration of bitter melon respectively), and found that it did impact glucose levels(30, 31). The water based extraction that was prepared (20mg/kg at 4.1% concentration) was found to be the dosage that reversed allcoxan induced hyperglycemia in rats with no toxicity to liver and kidneys for the 4 week experimental session. It was also determined that there was a 46% depression in plasma glucose as compared to the oral hypoglycaemic agent glibenclamide®(31). The power of this study raises issues on how representative these results are of the STZ rats used with respect to the overall population(30). Using a similar dosage, in a chronic study on allcoxan rats, (50, 100 and 200 mg/kg) MC was administered and showed a depression in blood glucose of 15.37%, 18.68% and 22.86% in plasma glucose levels on days 40, 50 and 60 respectively(32).



Human Studies

Research into the validity of the usage of MC on humans began in the later half of the 20th century. Leatherdale et al. were one of the first to study the effects of MC
on humans, and found that this herb lowered serum glucose considerably(33). As well, Welhinda and colleagues demonstrated that MC functioned to lower glucose in 73% of patients receiving it (see Figure 3). However, both studies only had a total of eight participants, which again raises issues of stastistical power due to such a small sample size(34).

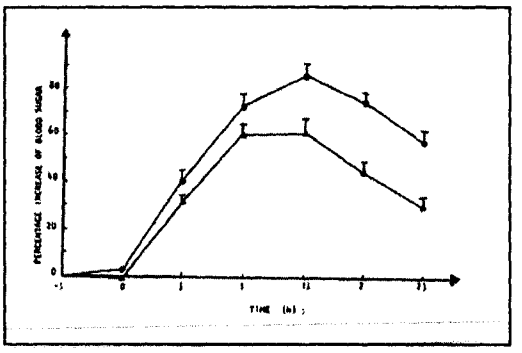


Figure 3: (Above) The pooled OGTT curves of diabetics patients *not responding* to MC whilst the test group (▲--▲) initially peaks but then decreases beyond that of control group (•---•). (Below) Diabetic patients responding to MC when given an OGTT. A marked difference can be observed between the control group (•---•), and

There are many ways of administering MC, including the juice of the fruit, powdered extract, and

the cooked vegetable form. Another study used diabetic patients who were taking an oral hypoglycaemic. These patients were administered (body weight (kg) * 2) a dosage, which was determined by the author of the study. No justification was given as to why this dosage was chosen, and therefore this brought questions of validity into consideration(35). Ultimately within these studies there are a multitude of issues which from a scientific perspective should have been taken into consideration. For example, the OGTT given in each of the trials was not standardized. Normally 75 g of glucose are given, in order to observe a change in levels of glucose, insulin and other related factors that are used to explore glucose homeostasis. Inclusion criteria and dosage are of main concerns to the patients. One study actually used diabetic patients, while on their oral hypoglycaemics, but did not control for this covariant via statistical analysis. action(31).

Rationale

Taken together, some, albeit limited, data suggest beneficial effects of *Momordica charantia* in diabetic individuals. However, further information in randomized placebo-controlled trials is needed. To our knowledge, the proposed study represents the first randomized placebo-controlled attempt to elucidate the acute effect of *Momordica charantia* on glucose homeostasis in humans. Another novelty of this research proposal is that the effects of MC on glucose control will be investigated in obese men. Obese individuals have high levels of fasting and postprandial glucose levels (18), rendering them more susceptible to develop type II diabetes. This research will also be the first to provide some information about the potential impact of *Momordica charantia* on circulating adiponectin levels, an adipose tissue-derived hormone regulating systemic insulin sensitivity. Finally, this research will be the first to explore the potential impact of *Momordica charantia* on the energy expenditure component in humans. Overall, the data obtained may yield a clearer understanding of how *Momordica charantia* influences glycemic control and body weight regulation, thus may have important implications for the potential use of *Momordica charantia* as a supplement for preventive and curative interventions in obesity and diabetes.

Objectives and Hypotheses: 1) To explore the acute effects of *Momordica charantia* on fasting and postprandial glycemia in obese men. We predict that *Momordica charantia* will improve fasting and postprandial glycemia. 2) To explore the acute effects of *Momordica charantia* on

circulating adiponectin levels. We predict that *Momordica charantia* will increase circulating plasma levels. 3) To explore the acute effects of *Momordica charantia* on energy expenditure. We predict that *Momordica charantia* will increase energy expenditure.

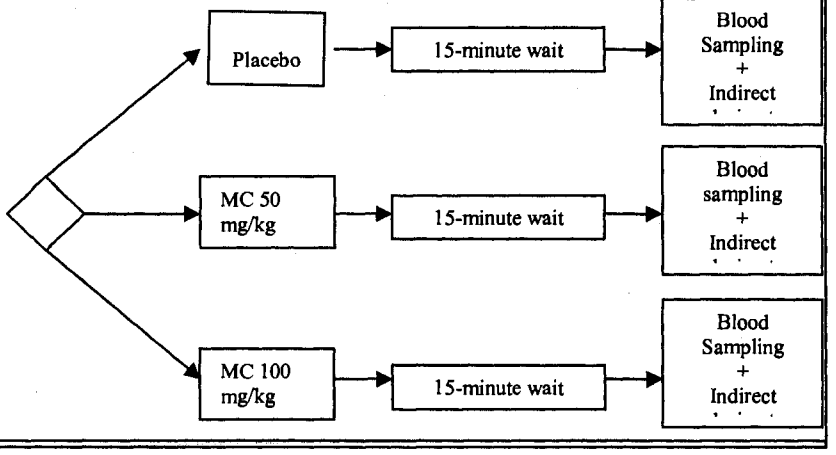
METHODS

Participants and Recruitment: The sample will be comprised of 24 obese men. Recruitment will be achieved by posting flyers at local universities. To encourage participation, a seminar will be given on lifestyle modification, as well as nutritional counselling following the clinical trial.

Inclusion Criteria. 1) Participants must be between the ages of 18-40; BMI of $\geq$ 27 kg/m^2; 2) Non-smoking; 3) Non-diabetic; 4) No thyroid based disorders, and or medication; 5) No excessive use of alcohol and or alcoholism or no current use of opiates.

Design: We will use a randomized, double-blind, placebo-controlled, cross-over laboratory study. Participants will visit our laboratory for one screening session and 3 experimental sessions. The study will be conducted within 1-year at the University of Ottawa.

Figure 1. Schematic diagram of the cross-over randomized trial.



Procedures. Interested participants will be screened via telephone initially to determine if they meet the criteria of the study. Eligible participants will then be asked to come to the laboratory for a pre-screening session where anthropometric variables (body weigh and composition (via a dual energy x-ray absorptiometer), height, waist circumference) will be measured. As shown in **Figure 1**, eligible subjects will undergo the following three experimental conditions in a randomized order: placebo, MC 50 mg/kg body weight and MC 100 mg/kg body weight freeze dried juice. The latter dosages of MC cover the approximate amount of MC extract previously given in the literature. Each of these conditions will be separated by a 2-week wash-out period to ensure that

no residual effects from the previous experimental session are carried forward. During each experimental session, the participants will be asked to fast for a period of 12 hours prior to arriving (7:45 am) at the laboratory. Participants will also be asked to refrain from any vigorous exercise 48 hours before experimental sessions and to refrain from consuming alcohol on the day prior to all experiments. Participants will also have to keep a record of their regular eating patterns in a 3-day journal before their first experimental session and maintain the same eating pattern prior to the 2 remaining experimental sessions. Once these recommendations respected, participants will be asked to lay down for a 10-minute resting period which will be followed by a 20-minute resting metabolic rate measurement by indirect calorimetery via a canopy system. The resting metabolic rate measurement will be followed by the insertion of a catheter in the antecubical vein by a registered nurse. The participant will then be asked to ingest the dosage of the supplement (8:30 am) which will be added to a standardized diet of 800kcal. For each experimental session, the participants will be provided with standardized breakfast (2 whole wheat slices of bread, 20g of Peanut Butter, 20g of Raspberry jam, 75g of Mild cheddar cheese and 250ml of Orange juice)

Quinine, which has a bitter taste, will be added to the placebo to ensure the participant is blindly consuming a placebo, 50mg/kg body weight or 100mg/kg body weight of freeze dried MC juice. The subject will be required to wait approximately 15 minutes after ingestion in order to ensure the absorption of the supplement. The placebo for this trial will be cellulose because it has no known activating properties on pancreatic function. Noteworthy is the fact that cellulose will also be included in the other experimental solutions. Blood samples during the OGTT will be collected in tubes through the catheter at -30,-15, 0, 30, 60, 90 and 120 minutes. Plasma insulin, glucose, C-peptide and adiponectin concentrations will be determined at each time point. Energy expenditure will also be estimated along the OGTT by indirect calorimetery by sampling expired air every 30-minute period.

Feasibility. We plan to run 3 new participants/month, and thus expect the experimental phase of the study to last about 7 to 8 months, leaving 4-5 months for data filing and statistical analyses. Our plan is to use approximately 24 subjects in this experiment. Given both the time and budgetary constraints of this strategic initiative, we believe that randomizing 24 subjects is a realizable objective that should give us sufficient statistical power (power of 0.80 with an alpha

of 0.05) to determine significance on a 15% difference at the 120-minute postprandial glucose levels between placebo and MC treatment (50/100 mg/kg).

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