THESIS

PHARMACOKINETIC INVESTIGATION OF COMMERCIALLY AVAILABLE EDIBLE MARIJUANA PRODUCTS IN HUMANS: POTENTIAL INFLUENCE OF BODY COMPOSITION AND INFLUENCE ON GLUCOSE CONTROL

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ABSTRACT

PHARMACOKINETIC INVESTIGATION OF COMMERCIALLY AVAILABLE EDIBLE MARIJUANA PRODUCTS IN HUMANS: POTENTIAL INFLUENCE OF BODY COMPOSITION AND INFLUENCE ON GLUCOSE CONTROL

Our investigation of five commercially available edible marijuana products containing 10mg of delta-9-tetrahydrocannabinol (THC) aimed to describe the pharmacokinetics of these products, investigate the potential influence of body composition on THC bioavailability, and, based on epidemiological research completed in the last decade, determine if acute marijuana ingestion influences glucose tolerance when compared to a THC-free gummy. We studied seven regular marijuana users. We utilized a single-blind randomized controlled crossover study design in which participants self-administered edible marijuana or a THC-free gummy. Thirty minutes following marijuana ingestion a standard oral glucose tolerance test was initiated via consumption of a 75g glucose drink. There was, at minimum, a four-day washout period between trials. Average time to peak plasma THC concentration ranged from 35 to 90 minutes, and average peak THC concentrations ranged from 3.2 to 5.5 ng/ml. Significant differences between products were identified twenty- and thirty-minutes post-ingestion. Several measures of body composition had significant correlations with plasma THC, although none of these correlations persisted across all products. There were no differences in indices of glycemic control between marijuana products or the THC-free gummy. Following acute edible marijuana ingestion in habitual users, significant differences in THC pharmacokinetics existed between similar products, possibly due to body composition, although glucose control was not impacted. In

summary, these data may inform recreational users to the proper dose for marijuana ingestion to achieve the desired outcome and to avoid overdose.

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TABLE OF CONTENTS

LITERATURE REVIEW

Introduction

The purpose of this review is to summarize previous research on Delta-9 Tetrahydrocannabinol (THC), the psychoactive component of *Cannabis sativa* L., pertaining specifically to absorption, metabolism, excretion, and overdose prevalence with a particular focus on edible marijuana. Then, in light of the epidemiological data suggesting that marijuana consumption may be beneficial for type 2 diabetes prevention, the review will provide a brief synopsis of type 2 diabetes (diagnoses, etiology, symptoms, and cost) and will finish with a brief overview of the data linking marijuana and type 2 diabetes and suggestions for future directions.

Background on Marijuana

Cannabis sativa L. is a plant originating in Asia and is currently grown in many places throughout the world[1]. It is commonly divided into two subcategories of plant: hemp, which contains little THC and is not psychoactive, or marijuana, which contains varying amounts of THC and can have psychoactive properties when consumed[2]. Since the 1970 Controlled Substances Act passed by Congress and signed into law by President Nixon, possession of marijuana is illegal under federal law. It is a misdemeanor first offense or felony second offense charge as it is a Schedule One drug: no current accepted medical use in the United States, a lack of accepted safety for use under medical supervision, and a high potential for abuse. Despite the federal prohibition of marijuana, many states have legalized marijuana for medical or personal use. California, in 1996, was the first state to legalize marijuana for medical use, with supporters citing the utility of marijuana for pain mitigation in people with acquired immunodeficiency syndrome (AIDS) or cancer. Several states followed, and in 2012 both Colorado and Washington

State legalized marijuana for recreational use. In 2014, the US Congress passed a bill prohibiting federal agencies from interfering with state medicinal cannabis laws. Currently, 18 states have legalized recreational use, and 35 states have legalized medicinal use, demonstrating an apparent conflict between marijuana's classification as a Schedule One illicit drug and the purported medicinal benefits. According to an April 2021 poll from Pew Research, 18% of American adults have used marijuana in the past year. State legislatures have recognized the utility of marijuana legalization. For example, since Colorado legalized marijuana in 2012, it has generated over $1.6 billion in state tax revenue, much of which goes to schools, drug awareness programs, or the state's general fund.

Background on THC

Marijuana is composed of numerous phytochemicals[3]. Perhaps the most familiar of these are cannabidiol (CBD) and THC. THC is the psychoactive component of marijuana that can produce an altered mood state, including euphoric or paranoid feelings via interaction with the endocannabinoid system. Specifically, THC crosses the blood-brain barrier and interacts with cannabinoid receptor 1 (CB1) to cause an inhibitory effect on neurotransmitter release and subsequent euphoria[4]. These receptors influence multiple neurotransmitters in the brain and are usually responsive to endogenous cannabinoids (endocannabinoids). The euphoria associated with marijuana use can be attributed to the more intense stimulation of CB1 receptors by THC compared with endocannabinoids. This review will primarily focus on edible marijuana, although differences in rate of absorption of ingested vs. inhaled marijuana will be discussed later.

THC Absorption, Metabolism, Storage and Clearance

The majority of ingested THC is absorbed from the small intestine[5]. However, unlike other forms of consumption (e.g., smoking), edible THC undergoes extensive first-pass hepatic metabolism (25-30%)[5,6]. Thus, the bioavailability of edible THC is likely to be significantly lower than inhaled or vaporized THC (dependent on numerous factors, including the size of inhalation or duration of breath-hold). In addition to lower bioavailability, edible THC takes longer to become bioavailable when compared to inhaled THC. For example, maximal THC concentration in the bloodstream following inhalation may occur nine minutes post inhalation, whereas edible THC may take up to five hours before it reaches maximal concentration[7]. Further, ingested THC is also cleared from the blood much more slowly than inhaled THC, potentially due to prolonged entry of THC due to gastric emptying and absorption from the small intestine (i.e. akin to bolus *vs*. prolonged administration). One possible implication of these findings is that edible THC may be the preferred application method for medical treatments involving chronic pain, considering the longer lasting and more consistent blood THC concentration following ingestion compared to inhalation.

THC is primarily metabolized in the liver by Cytochrome P450 (CYP450) proteins. THC is broken down into 11-Hydroxy-THC (THC-OH) and 11-Nor-9-carboxy-THC (THC-COOH) and then undergoes glucuronidation and excretion[8]. While the liver is the primary location for THC breakdown, it can also be broken down via the CYP450 proteins in the small intestine[9] (influencing bioavailability) or other sites with CYP450 proteins.

Once in the bloodstream, THC is bound to red blood cells (10%) or travels within plasma (90%)[10]. THC in plasma is attached primarily to lipoproteins, although some may be carried by albumin. THC distributes to the most highly vascularized locations first and is distributed from blood to deeper tissue as the concentration of THC and the equilibrium constant allows. THC is lipophilic and thus can rapidly be transported (via fatty acid binding proteins) through cell membranes and into tissue[7]. Additionally, due to THC's lipophilic nature, a significant amount of THC may be stored in the adipose tissue of chronic users and may be re-released when lipolysis occurs, causing reintoxication[11]. Due to the rapid disbursement of THC and its lipophilic nature, total elimination of THC may take weeks following abstention as adipose or other deep compartments can continue to provide a steady supply of THC to the blood pool to maintain equilibrium between plasma and tissue[8].

Determinants of THC Absorption

Multiple factors may influence the absorption and bioavailability of edible THC products. The composition of the edible itself may accelerate or hinder digestion. As THC is lipophilic, THC that is bound to or consumed with a medium-chain triglyceride (MCT) oil may be more rapidly absorbed via co-transport with MCTs through the portal vein and into circulation once past first-pass metabolism[12]. There also may be differences between THC ingested when either in a fed or fasted state. As THC is generally well absorbed in the small intestine, a fed state may provide a more consistent, steady influx of THC into the blood as food influences the rate of gastric emptying. Additionally, a high fat meal may assist with absorption and co-transport of the THC. However, contrary to THC consumption in the fed state, users of edible cannabis that are fasted will experience a more rapid time to peak concentration and subsequent decrease in plasma

THC, and the total area under the curve (AUC) of plasma THC may be lower [13]. This could be due to food influencing the rate of gastric emptying and the influence of fatty acids on THC transport. A person in the fasted state will immediately uptake and subsequently metabolize THC, but total THC concentrations (as measured by the plasma THC AUC), may be lower due to a lack of food to act as a co-transporter and thus lesser THC absorption from the gut.

Regular THC users have a greater rate of hepatic THC metabolism than THC naïve individuals, presumably due to the upregulation of CYP450 proteins[5]. It is currently unknown whether circulating THC concentration is influenced by body composition. However, a recent study investigating the pharmacokinetics of another cannabinoid, cannabidiol, suggested that fat-free mass was a significant predictor of time to peak concentration of the formulation with the greatest bioavailability, although the correlation was negative and the peak may have been induced by a more rapid clearance of CBD rather than a clinically significant boost in bioavailability[14]. Theoretically, individuals with a greater fat mass may experience less psycho-activity, as a more significant proportion of THC is diverted to adipose tissue rather than brain tissue. On the other hand, well-trained individuals may have greater vascularization within skeletal muscle and thus more THC is diverted from the brain. More studies are required to determine if body composition is a source of inter-individual variability in THC pharmacokinetics, which will help determine the minimum effective dose to receive the desired recreational or medicinal effects.

Determinants of the Time-Course of THC

Sensation kinetics of THC are based on several different factors. One such factor is the method of consumption. Users who consume THC via inhalation will perceive psychoactive effects nearly immediately, and the effects last for up to 45 minutes. On the other hand, edible THC can take much longer before an effect is felt (45 minutes – 2 hours before any psychoactive impact is perceived)[5]. Interestingly, 11-THC-OH, one of the metabolites of THC through first and subsequent pass hepatic metabolism, potentially has a greater capacity to cross the blood-brain barrier and thus may be more psychoactive than THC, and may also extend the psychoactive influence of the drug[8,15]. As edible THC starts with hepatic metabolism, psychoactive effects may be felt for an extended time as both the THC from consumption and the 11-THC-OH from first-pass metabolism can all contribute to the bioactivity of the drug. Additionally, as THC is rapidly distributed from plasma to tissue, to maintain equilibrium, some users may get a second peak in plasma concentrations of THC after a couple of hours as tissue redistributes back to plasma via passive diffusion[11] when plasma concentration is low[7]. Regular users of cannabis can develop a tolerance due to saturation or down-regulation of the CB1 receptors, thus requiring a continually higher dose to retain the desired psycho-activity, or regular periods of abstention (i.e. "tolerance breaks")[16].

Prevalence of Overdose

A retrospective analysis from January 2013 to December 2015 noted 430 calls to the poison control center pertaining to edible marijuana consumption. Five of these 430 calls were medically coded as "major effect", with symptoms consisting of respiratory arrest, seizures, hallucinations, chest pain, and/or others. One hundred forty-one calls were coded as "moderate

effect" and 247 as "minor effect"[17]. Of note, these data were collected retrospectively and via the poison control center database and thus may underrepresent other cases of a marijuana overdose not reported to the poison control center. These data are also not reflective of the additional states that have newly permitted recreational or medical marijuana use since 2016.

There are several potential symptoms of a THC overdose. One such symptom is anxiety. Rodent studies evaluating the influence of THC on anxiolytic or anxiogenic effects determined that higher doses of THC are correlated with anxiogenic outcomes, primarily due to the influence of the activation of CB1 receptors in the amygdala[18]. Another symptom of THC overdose may be cardiac complications, such as hypo- or hyper-tension or tachycardia[19]. This apparent conflict in outcomes may be explained due to varying mechanisms of THC action. THC may cause hypotension due to its role as a vasodilator or may cause hypertension due to its influence on sympathetic outflow. Two explanations exist for this apparent paradox: 1. THC can cause vasodilation within the systemic circulation, thus causing reflex tachycardia, in which the heart beats more rapidly in response to a reduction in blood pressure in attempt to maintain blood flow. This is supported by evidence showing THC use may cause orthostatic hypotension and supine hypertension. 2. THC can modulate sympathetic nervous system activity and thus increase heart rate, or, independent of the sympathetic nervous system, promote automaticity within cardiac cells leading to tachycardia or even atrial fibrillation[20]. Loss of respiratory drive has also been reported[17]; the mechanisms are unclear but may pertain to the signaling from the CB1 receptors that line the brainstem or the potential for THC to inhibit the dorsal vagal nucleus, a structure within the brain responsible for vagal innervation in the lungs[21].

There are currently few studies that describe the pharmacokinetics of ingested THC. This is a problem, as many of the side effects listed above are related to overconsuming THC, either via accidental/unintentional ingestion, or premature repeated doses. The latter may be prevented by providing marijuana users with detailed information pertaining to THC pharmacokinetics, including maximal circulating concentration (C_{Max}) and time to maximal concentration (T_{Max}). Additionally, there are multi-variate factors that may potentially influence the circulating concentration of THC. For example, there are no data on how body composition may influence the response to a standardized dose of THC. Although it can reasonably be surmised that a sedentary male with obesity may have a different response than a lean, athletic female, there is currently no recommendation for either of those populations, or available to their physicians to refer when attempting to ascertain an appropriate dose for medicinal needs. In addition to body composition, age may influence optimal dosing strategy. An older adult may not be able to metabolize THC as well due to decreased hepatic clearance of drugs[22], and thus a lower dose may be recommended. Similarly, differences in health, such as sympathetic nervous system responsiveness, liver health, cardiovascular function, or potential disease states, may also influence the optimal dose.

Additionally, the THC formulation likely influences bioavailability. Modern technology has improved the formulation of products such that equivalent doses may provide different responses based on varied/improved delivery technology. While it appears that the fed state provides a more consistent and even dose of THC, there are no data available to suggest on which platform the THC should be delivered. Cookies, gummies, tinctures (THC “oil” with an alcohol base), or a sublingual powder (powder dissolved under the tongue) all may have very different

pharmacokinetic properties. There is a critical need for more research in this area, as a one-size-fits-all approach can lead to overdose even if the THC concentrations are identical.

While the potential effects of an overdose are serious, there are also data to suggest that THC can provide health benefits *if dosed properly*. THC containing drugs (Dronabinol and nabilone) are currently both FDA approved for treatment of emesis attributed to chemotherapy, and anorexia. Additionally, epidemiological research suggests that marijuana may be useful to prevent the development of type 2 diabetes[23].

Overview of Diabetes

Diabetes is a glucose regulation disorder that is diagnosed in a few different ways. These include glycated hemoglobin (HbA1c) greater than or equal to 6.5% on repeated tests, a random blood glucose test indicating concentration >200mg/dL, fasting glucose concentration >126mg/dL, or an Oral Glucose Tolerance Test (OGTT) that results in a blood glucose concentration > 200mg/dL after 2 hours[24].

Excessive caloric intake can lead to a loss of insulin sensitivity, followed by an attempt by the pancreas to compensate by increasing the production of insulin. This then causes further insulin receptor resistance and eventually can lead to beta-cell failure[25] and type 2 diabetes. Beta-cell insufficiency is also associated with Type 1 diabetes but is caused by a susceptible genotype and environmental factors rather than long term excess caloric intake. Type 1 diabetes is considered a hereditary auto-immune[26] disorder, whereas type 2 diabetes is considered an acquired disease

due to lifestyle. This review will focus on the latter, since there is evidence that type 2 diabetes can be "reversed" or put into remission with medical or lifestyle interventions[27].

Etiology of Type 2 Diabetes

There are several theories as to the etiology of type 2 diabetes. The simplest is that consistently high post-prandial blood glucose and the subsequent insulin response causes a down-regulation of the cellular insulin receptors; requiring a higher insulin load to meet the threshold for insulin to activate the protein signaling cascade to promote Glucose Transporter 4 (GLUT4) translocation and subsequent glucose shuttling into the skeletal muscle[28]. A second theory revolves around intramuscular triglycerides (IMTGs) and the potential interference with the insulin signaling cascade. IMTGs that are not fully metabolized, such as ceramides and diacylglycerols, interfere with the insulin signaling cascade. Ceramides cause serine phosphorylation instead of tyrosine on the Insulin Receptor Substrate 1[29], preventing the signaling cascade from continuing, whereas diacylglycerols inhibit phosphoinositide 3-kinase[30] from activating protein kinase B to cause the translocation of GLUT4 to the cell membrane and allowing glucose to enter the muscle.

In either case, it appears that long-term excessive caloric intake and a lack of exercise are the primary drivers of the development of type 2 diabetes[31]. Treatments can include non-pharmaceutical interventions, such as weight loss or commencement of a structured exercise program, as well as pharmaceutical interventions, such as Metformin, Sodium-Glucose Transporter 2 inhibitors, GLP-1 agonists, and others. These interventions reduce the glucose

load, stimulate insulin release, help increase glucose disposal, and/or increase energy expenditure.

Symptoms of Type 2 Diabetes

Diabetes alone is the 7th leading cause of death in the United States[32] and is also implicated in the etiology of other diseases, such as cardiovascular disease[33]. Additionally, Type 2 Diabetes is the leading cause of blindness[34] and non-traumatic limb amputation[35]. There is also an increased risk for the development of brain diseases such as stroke[36] or Alzheimer's[37]. Type 2 diabetes also causes a reduction in exercise tolerance[38], perhaps due to a diminished oxygen off-loading capacity[39] or microvascular impairment leading to decreased oxygen uptake. Type 2 diabetes may also lead to diabetic ketoacidosis, a life-threatening condition in which excessive ketone bodies are produced in an attempt to fuel cells unable to utilize blood glucose.

Type 2 diabetes also leads to less (relatively) serious quality of life issues, such as excessive tiredness, extreme hunger, excessive thirst, increased sickness or infection, or frequent urination. Additionally, a reduced oxygen-carrying/utilization capacity may reduce maximal oxygen utilization below a functional living threshold[40] and cause premature assignment to a facilitated care facility, such as a nursing home.

Cost of Type 2 Diabetes

34.2 million (1 in 10) Americans have diabetes[41]. In comparison, this is just slightly less than the number of Americans who had and recovered from COVID-19. However, Type 2 diabetes is

considered a lifestyle disease and is not caught via contagion. In comparison to other serious diseases, this is more than five times the number of Americans living with Alzheimer's disease (6 million)[42], and nearly double the number of Americans with coronary artery disease (18 million)[43]. Three hundred and twenty-seven billion dollars, or $1 of every $7, is spent on healthcare costs related to diabetes[44]. This is a more significant financial burden than Alzheimer's disease, heart disease, or cancer. The prevalence of diabetes is expected to skyrocket in upcoming decades, with projections predicting an increase of adults with Type 2 diabetes reaching 61 million in 2060[45]. An individual with type 2 diabetes can expect to spend 2.3 times more in healthcare costs than they would have spent without the disease[44].

Marijuana and Type 2 Diabetes

Epidemiological research published in the last decade suggests that regular marijuana use may be protective against type 2 diabetes, even when accounting for covariates such as sedentariness or obesity. Cross-sectional studies have determined that marijuana users were less likely to have obesity than non-users. Additionally, mean body mass index was lower in current users compared to non-users[23]. However, these data relied on self-report and thus may be prone to response bias (inaccurate reporting to maintain social status or due to fears of retaliation due to the illicit nature of marijuana). A different study utilized National Health and Nutrition Examination Survey (NHANES) data from 2005-2010 that included self-report of marijuana use and clinical tests of fasting insulin and glucose and found that current marijuana users had 16% lower fasting insulin levels and a smaller waist circumference. This study also controlled for numerous potential confounding variables, including age, sex, tobacco and alcohol use, and activity status[46], although marijuana use was self-reported. Another study utilizing more recent

NHANES data (2009-2016) ran a similar analysis but separated participants by weight category: lean, overweight, and obese, and found that within the obese category, compared with adults who had never used marijuana, fasting insulin was lower in current marijuana users, irrespective of frequency of use (i.e. less than 4/month vs. more than 8/month). Former users who quit marijuana between one and 10 years prior to the study also exhibited lower fasting insulin, although to a lesser extent, suggesting that the protective effect wanes through time post-cessation[47].

The endocannabinoid system is implicated in obesity. Individuals with obesity have an overactive endocannabinoid system and THC consumption impairs glucose tolerance in both rodents and humans. One theory explaining the paradoxical relationship between marijuana use and protection against diabetes is chronic marijuana usage may down-regulate the CB1 receptors that are implicated in obesity. Thus, marijuana may not be protective because of its direct actions on CB1 receptors, but rather the downstream influence of CB1 down-regulation[48].
Aside from three small studies completed in the 1970's, there have been no studies directly addressing the question of acute marijuana use on glucose tolerance. One study reported that marijuana inhalation impaired glucose tolerance (n = 4)[49]. Another study (n = 6) also reported a glucose impairment with intravenous THC administration. The final study (n=10) utilized smoke inhalation during the oral glucose tolerance test and found no influence of marijuana on glucose tolerance when compared to placebo[50].

With 192 million worldwide users of cannabis[51], and with more people beginning to use as legalization relaxes[52], there is a critical need to determine the pharmaceutical and

pharmacokinetic properties of this drug. There are mutually exclusive ideas as to the safety of the drug. For example, the reports of potential cardiovascular complications seem to conflict with reports considering THC as a therapy for high blood pressure. The evidence for or against marijuana use appears piecemeal, and more work must be done to provide a clearer picture of the future of marijuana research. Ideally, future directions would include legalization or a research exemption to allow scientists to run well-designed randomized controlled trials. In the absence of such, scientists can complete observational studies, such as one recently undertaken at the University of Colorado at Boulder in which participants self-administered marijuana and then were available for testing[53]. Additionally, epidemiologists can continue to identify trends in health outcomes (positive or negative) in marijuana users that can be then mechanistically studied using model systems to provide hypothesis as an impetus to make human marijuana research more accessible to scientists. Marijuana works within an endogenous system and there is clear evidence that it modulates physiology in a variety of ways. As such, and considering the widespread prevalence of use, there is a clear and vital public health interest in continued study.

INTRODUCTION

Cannabis sativa L. is a plant originating in Asia and is currently grown in many places throughout the world[1]. It is commonly divided into two subcategories of plant: hemp, which contains little delta-9 tetrahydrocannabinol (THC) and is not psychoactive, or marijuana, which contains varying amounts of THC and can have psychoactive properties when consumed[2]. In the USA, marijuana consumption is currently a federal offense, although many states have passed legislation allowing for medical or recreational use. In conjunction with these legalization efforts, the prevalence of marijuana use, and subsequent overdose, has increased. While acute marijuana overdose is unlikely to be lethal, it can lead to a variety of unintended consequences ranging from mild to severe, such as increased anxiety/paranoia[52], orthostatic hypotension[19], or respiratory depression[17]. One potential explanation for increased prevalence of overdose is the varying potency of products based on delivery method. For example, edible brownies containing 50mg THC produced a maximal concentration (C_{Max}) of 2.5ng/ml to 4ng/ml[54], whereas a 5.9% THC cigarette produced a C_{Max} of 28.3ng/ml[55]. Body composition may also play an important role in THC concentration. A study evaluating the bioavailability of a different cannabinoid, cannabidiol (CBD), found that C_{Max} was related to fat free mass[14]. However, due to marijuana's Schedule One drug status, clinical trials evaluating THC pharmacokinetics are scarce. This has public health relevance, as marijuana use increases as legalization relaxes[52]. With greater prevalence of use, THC overdose will become more rampant until pharmacokinetic data can inform public health safety messaging.

On the other hand, epidemiological research published in the last decade suggests that regular marijuana use may be protective against type 2 diabetes, even when accounting for covariates such as sedentariness or obesity. Cross-sectional studies have determined that marijuana users were less likely to have obesity than non-users. Additionally, mean body mass index was lower in current users compared to non-users[23]. However, these data relied on self-report and thus may be prone to response bias (inaccurate reporting to maintain social status or due to fears of retaliation due to the illicit nature of marijuana). A different study utilized National Health and Nutrition Examination Survey (NHANES) data from 2005-2010 that included self-report of marijuana use and clinical tests of fasting insulin and glucose and found that current marijuana users had 16% lower fasting insulin levels and a smaller waist circumference. This study also controlled for numerous potential confounding variables, including age, sex, tobacco and alcohol use, and activity status[46], although marijuana use was self-reported. Another study utilizing more recent NHANES data (2009-2016) ran a similar analysis but separated participants by weight category: lean, overweight, and obese, and found that within the obese category, compared with adults who had never used marijuana, fasting insulin was decreased in current marijuana users, irrespective of frequency of use (i.e. less than 4/month vs. more than 8/month). Former users who quit marijuana between one and 10 years prior to the study also exhibited lower fasting insulin, although to a lesser extent, perhaps suggesting that the protective effect, if initially detected, wanes through time post-cessation[47]. These data support a hypothesis that marijuana use may be protective against type 2 diabetes, although epidemiological studies cannot support causation and clinical trials are required to fully elucidate the potential connection between marijuana use and type 2 diabetes prevalence.

In this study, we evaluated the pharmacokinetics of five different commercially available edible marijuana products all standardized to a 10mg THC dose. Our aims were to describe the pharmacokinetics of these products, with special attention towards varying pharmacokinetic parameters between products despite a standardized dose, to evaluate the potential influence of body composition on the pharmacokinetics of THC and its metabolites (11-Hydroxy-THC (THC-OH) and 11-Nor-9-carboxy-THC (THC-COOH), and, considering the epidemiological link between marijuana use and type 2 diabetes, to determine the influence of THC on blood glucose in response to an oral glucose tolerance test. We hypothesized that 1. There would be no difference in THC pharmacokinetics of varying formulations (although all edible and from the sativa strain of the marijuana plant) standardized to a 10mg dose, 2. Body composition would correlate with pharmacokinetic parameters related to THC, and 3. Marijuana ingestion would lead to favorable modification of glycemic control when compared to a marijuana-free control product.

METHODS

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of Colorado State University (Protocol #20-10278H, 8 October 2020). All participants provided written informed consent prior to commencement of the study. This study utilized a randomized, repeated measures crossover design.

Participants

Adult men and women aged 21 or over were invited to participate. Inclusion criteria included body mass greater than 50 kg, regular use of marijuana (≥four times in the previous month), willingness to abstain from all products derived from *Cannabis sativa* L. during the four days prior to each data collection, and previous use of a *Cannabis sativa* L. product containing ≥10 mg of THC without a significant adverse reaction. Exclusion criteria included pregnancy, breastfeeding, treatment for psychosis, bipolar disorder or schizophrenia, current or previous use of medication for treatment or prevention of diabetes, previous diagnoses of heart disease, peripheral vascular disease, high blood pressure, stroke, or heart murmur, and/or use of medication contraindicated for concurrent use with marijuana or known to influence glycemic control.

Protocol Overview

To remain compliant with institutional and state laws pertaining to marijuana, an

observational approach was employed in a manner inspired by a recent study conducted by the University of Colorado at Boulder, in which the participants purchased and self-administered marijuana[53]. Following screening, on six mornings, each separated by a minimum of four-days, participants self-administered one of five edible marijuana products or a marijuana-free control product in a randomized crossover design; each marijuana product contained 10 mg of THC. Thirty minutes following marijuana (or marijuana-free control) ingestion, participants consumed a carbohydrate beverage containing 75 g of glucose. Venous blood was sampled repeatedly over 4-h and was analyzed for circulating concentrations of THC, THC-COOH, THC-OH, glucose, and insulin.

Procedures

Prior to study enrollment, potential participants completed a detailed electronic medical history questionnaire. Responses requiring additional query were addressed either in-person, via telephone, or video conference. Body size and composition were assessed at Colorado State University using dual energy X-ray absorptiometry (DEXA; Hologic, DiscoveryW, QDR Series, Bedford, MA, USA), and a physician's digital scale and stadiometer.

The remaining data collection sessions were completed off-campus on six mornings, each separated by 1–2 weeks depending on participant schedules. The time of protocol initiation was kept constant for each participant. Every data collection was preceded by a 12-h fast, 24-h abstention from alcohol and exercise, and 96-h abstention from any products derived from *Cannabis sativa* L., including CBD and marijuana.

A venous catheter was introduced to an antecubital or dorsal hand vein and blood (~10 mL) was collected for analysis of baseline circulating concentrations of THC, THC-COOH, THC-OH, glucose, and insulin. Immediately following baseline blood collection, participants self-administered one of five edible marijuana products or a marijuana-free control product (described in detail in a subsequent section). Thirty minutes following marijuana (or marijuana-free control) ingestion, participants consumed a beverage consisting of 75g of glucose dissolved in 250 mL of water (i.e., an oral glucose tolerance test (OGTT)).

Relative to marijuana ingestion (Time 0), venous blood was sampled for subsequent analysis of circulating concentrations of THC, THC-COOH, and THC-OH at minutes 10, 20, 30, 45, 60, 75, 90, 120, 180, and 240. Blood was immediately transferred into chilled tubes coated with ethylenediaminetetraacetic acid (K3 EDTA) and placed on ice for up to 30 min before isolation of plasma via chilled (4C) centrifugation. One milliliter aliquots of plasma were then placed on ice while being transported to the research facility for storage at -80C prior to subsequent analysis.

Relative to marijuana ingestion (Time 0), the carbohydrate beverage was consumed at minute 30. Venous blood was sampled for subsequent analysis of circulating concentrations of glucose at minutes 25 (i.e., post-marijuana but pre-glucose), 35, 40, 45, 50, 60, 75, 90, 105, 120, 135, 150, 180, and 240, and at minutes 25, 45, 75, 105, 135, and 150 for subsequent analysis of plasma insulin concentration. Blood intended for glucose analysis was transferred to chilled tubes containing sodium fluoride (potassium oxalate), and then

immediately placed on ice for transport to the research facility where it was evaluated, in duplicate, without delay using an automated analyzer (YSI 2900 STAT Glucose Lactate Analyzer, YSI Inc., Yellow Springs, OH, USA). Blood intended for insulin analysis was processed in an identical manner to the blood used for THC, THC-COOH, and THC-OH analysis. Plasma insulin concentration was determined in triplicate via enzyme-linked immunosorbent assay ((ELISA) Crystal Chem, Inc., Elk Grove Village, IL, USA).

Commercially Available Edible Marijuana Products

Five commercially available edible marijuana products were selected for study. These products were made and sold at licensed stores throughout Colorado, USA. Participants were requested to purchase each of the identified products using their personal funds. Confirmation of purchase was verified by inspection of retail receipt. Features of each of the edible marijuana products are presented in Table 1. Each of the products contained 10 mg of THC, except for the marijuana-free control product. The marijuana-free control product was provided by the research team (i.e., purchase by participants was not required). All products were consumed within 30 s of self-administration. The order of self-administration was dictated by the research team based on a random generator.

Table 1. Features of the commercially available edible marijuana products.

Product and Manufacturer	Nutrition	Ingredients
Ripple Blood Orange Gummies (Stillwater Brands, Commerce City, CO, USA)	20 kcal per serving: 2 gummies; (Fat 0 g, Total carbohydrate 4 g, Protein 0 g)	Glucose syrup, sugar, water, fruit juice concentrates (Apple, Pear), gelatin, modified food starch, Ripple (water, modified food starch, cannabinoid extracts, MCT oil), contains 2% or less of: natural flavors, malic acid, citric acid, carnauba wax, vegetable juice for color.

Ripple Pure 10 (Stillwater Brands, Commerce City, CO, USA)	0 kcal per serving (Fat 0 g, Total carbohydrate 0 g, Protein 0 g)	Sorbitol, modified food starch, cannabinoid extracts, MCT oil
Ripple Quick Sticks Blueberry Pomegranate (Stillwater Brands, Commerce City, CO, USA)	5 kcal per individual serving (Fat 0 g, Total carbohydrate 1 g, Protein 0 g)	Ripple (Sorbitol, Modified Food Starch, Cannabinoid Extracts, MCT Oil), Sugar, Fructose, Natural Flavors, Citric Acid, Malic Acid
Wana Fast Acting Gummies, Pina Colada Indica (Wana Brands, Boulder, CO, USA)	30 kcal per serving: 2 gummies (Fat 0 g, Total carbohydrate 8 g, Protein 0 g)	Organic Cane Sugar, Organic Tapioca Syrup, Pectin (Pectin, Potassium Sodium Tartrate, Polyphosphate, Sucrose), Citric Acid, Natural Flavoring, Sodium Citrate, Modified Food Starch, Xanthan Gum, THC.
Wana Sour Gummies (Wana Brands, Boulder, CO, USA)	15 kcal per serving (Fat 0 g, Total carbohydrate 4 g, Protein 0 g)	Organic Sugar, Organic Tapioca Syrup, Pectin (Pectin, Potassium Sodium Tartrate, Polyphosphate, Sucrose), Citric Acid, Natural Flavoring and Coloring, Sodium Citrate, Marijuana Concentrate, and Botanical Terpenes for Flavor.
Welch's Fruit Snacks (Park Ridge, NJ, USA) *	15 kcal per serving (Fat 0 g, Carbohydrate 2 g, Sugar 3 g, Protein 0 g)	Fruit puree (grape, peach, orange, strawberry, and raspberry), corn syrup, sugar, modified corn starch, gelatin, concord grape juice from concentrate, citric acid, lactic acid, natural and artificial flavors, ascorbic acid (vitamin C), alpha tocopherol acetate (vitamin E), vitamin A palmitate, sodium citrate, coconut oil, carnauba wax, annatto (color), turmeric (color), red 40, and blue 1.

All self-administered doses of edible marijuana products contained 10 mg THC. * Marijuana-free control product. MCT: Medium chain triglycerides.

Reagents and Supplies

THC, THC-COOH, THC-OH, THC-D3, THC-COOH-D3, and THC-OH-D9 were purchased from Cerilliant (Round Rock, TX, USA). A second set of THC, THC-OH, and THC-COOH was purchased from Lipomed (Cambridge, MA, USA) to be used for quality control samples. Water and acetonitrile (LC–MS-grade) were obtained from Millipore

(Burlington, MA, USA). Dansyl chloride, sodium bicarbonate, sodium carbonate, acetic acid, and formic acid (LC–MS-grade) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Captiva EMR-Lipid columns (1 mL, 40 mg) were purchased from Agilent Technologies (Santa Clara, CA, USA). Chromatography was performed with a Kinetex Phenyl Hexyl column (3.0 x 50 mm, 2.6 **µ**m) purchased from Phenomenex Inc. (Torrance, CA, USA).

Calibrators, Quality Controls, and Internal Standard Preparation

Matrix matched calibrators and controls were prepared by the addition of appropriate volumes of methanolic stock standard mixes to 300 **µ**L of cannabinoid free plasma. Working standard mixes containing 0.01, 0.1, or 1.0 **µ**g/mL of THC, THC-OH, and THC-COOH were prepared from stock standards obtained from Cerilliant. They were used to produce calibrators for THC and THC-OH at 0.05, 0.1, 0.2, 0.5, 1, 5, 10, and 50 ng/mL and calibrators for THC-COOH at 0.2, 0.5, 1, 5, 10, and 50 ng/mL. Quality control samples were prepared at 0.7, 7 and 20 ng/mL for each analyte using working standard mixes of 0.01, 0.1 or 1.0 **µ**g/mL of THC, THC-OH and THC-COOH. These working standards were prepared from stock standard obtained from Lipomed to verify the calibrators prepared from Cerilliant stock standards. Quality control samples were run after every 20 subject samples with an expected accuracy of +/-20%. The internal standard mix solution contained 30 ng/mL THC-D3, 100 ng/mL THC-OH-D3, and 300 ng/mL THC-COOH-D9 in methanol.

Cannabinoid Analysis by LC-MS/MS

Plasma samples and matrix-matched standards and quality controls were prepared for LC-MS/MS analysis by protein precipitation, lipid removal, and derivatization with dansyl

chloride. Ten microliters of internal standard solution were added to 300 µL of plasma sample and mixed in a microcentrifuge tube. Nine-hundred microliters of acetonitrile containing 1% formic acid were added and vortexed for 30 s to precipitate proteins. Samples were centrifuged and supernatants transferred to Captiva EMR-Lipid columns for lipid removal. Using a positive pressure manifold, 3 psi of pressure was applied to the samples to elute through columns. Eluents were collected into a clean glass test tube and dried under nitrogen at 40 C prior derivatization. Dried eluents were reconstituted in 100 µL of 1 mg/mL dansyl chloride in acetonitrile and transferred to autosampler vials fitted with 400 L glass inserts. One-hundred microliters of a 0.1 M sodium carbonate bicarbonate buffer (pH 10) were added and the sample incubated at 55C for 20 min to derivatize the analytes. Samples were cooled to room temperature and neutralized with 10 µL of acetic acid prior to LC-MS/MS analysis.

Samples were analyzed with an Agilent 1290 UHPLC coupled to an Agilent 6460 triple quadruple mass spectrometer equipped with an Agilent Jet Stream electrospray ionization source (Agilent, Santa Clara, CA, USA). Cannabinoids were first chromagraphically separated on a Phenomenex Phenyl Hexyl column (3.0 x 50 mm, 2.6 µm) held at 40 C. A sample volume of 10 µL was injected and a mixture of water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B) at a flow rate of 0.4 mL/min. The gradient elution used was 40% B for 0.5 min, increasing to 80% B at 2 min, increasing to 100% B at 4.5 min, and held at 100% B for 1.5 min. The ionization source conditions used were as follows: positive polarity, nebulizer 45 psi; gas flow of 10 L/min at 300 C; sheath gas flow of 12 L/min at 390 C; capillary voltage of 3500 V; nozzle voltage of 200 V. The ion

transitions monitored are displayed in Table 8. Analytes were confirmed by retention time and the product ion ratio correlation between the sample peaks and corresponding standards (±20%). The data collection and processing were performed by using Agilent MassHunter Quantitative software (v.B.08.01). Quantitation was performed with linear regression using 8-point calibration curves from 0.05 ng/mL to 50 ng/mL for THC and THC-OH. A 6-point calibration curves from 0.2 ng/mL to 50 ng/mL was used for THC-COOH. Analytical staff were naïve as to the edible marijuana products (i.e., blind to specific products and conditions).

Pharmacokinetic and Oral Glucose Tolerance Test Analysis

Pharmacokinetic analysis of the circulating concentrations of THC, THC-OH, and THC-COOH for each of the products was completed using dedicated software (PhoenixWin-Nonlin v8.3, Certara, NJ, USA). Areas under the concentration curves were calculated using the linear trapezoidal method.

Glucose and insulin data were processed using established methods. These included calculation of Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) and Matsuda Index. Areas under the concentration curves for 2-h (standard practice) and for 3.5 h (practice specific to the current study) were calculated using the linear trapezoidal method.

Statistical Analyses

All data, unless otherwise stated, are expressed as mean and standard deviation.

Statistical calculations were performed using dedicated software (SigmaStat 3.0, Systat Software Inc., San Jose, CA, USA). Differences in circulating concentrations of THC, THC-OH and THC-COOH, glucose, and insulin over time and between products were examined using 2-way analysis of variance (ANOVA; product x time), with repeated measures (time). Differences in the pharmacokinetic properties between the edible marijuana products were examined using 1-way ANOVA, with repeated measures. When criteria for parametric statistics were not satisfied (i.e., normality and equal variance), a nonparametric alternative, Friedman Repeated Measures ANOVA on ranks, was used. Tukey tests were employed to further interrogate identified main effects. Relations between THC pharmacokinetic parameters and body size and composition values were explored using Pearson correlations. The level of statistical significance was set at $p < 0.05$.

RESULTS

Fifteen people consented to participation in our study. Eight participants withdrew from participation early, due to issues with phlebotomy (n = 2), a broken ankle (unrelated to our intervention) requiring surgery (n = 1), inability to tolerate the research protocol (n = 4), or inability to abstain from marijuana use between visits (n = 1). Seven participants completed every study visit and were used for analysis. Details are provided in Figure 1 (Consolidated Standards of Reporting Trials (CONSORT) diagram). Table 2 depicts baseline characteristics of the seven included study participants.

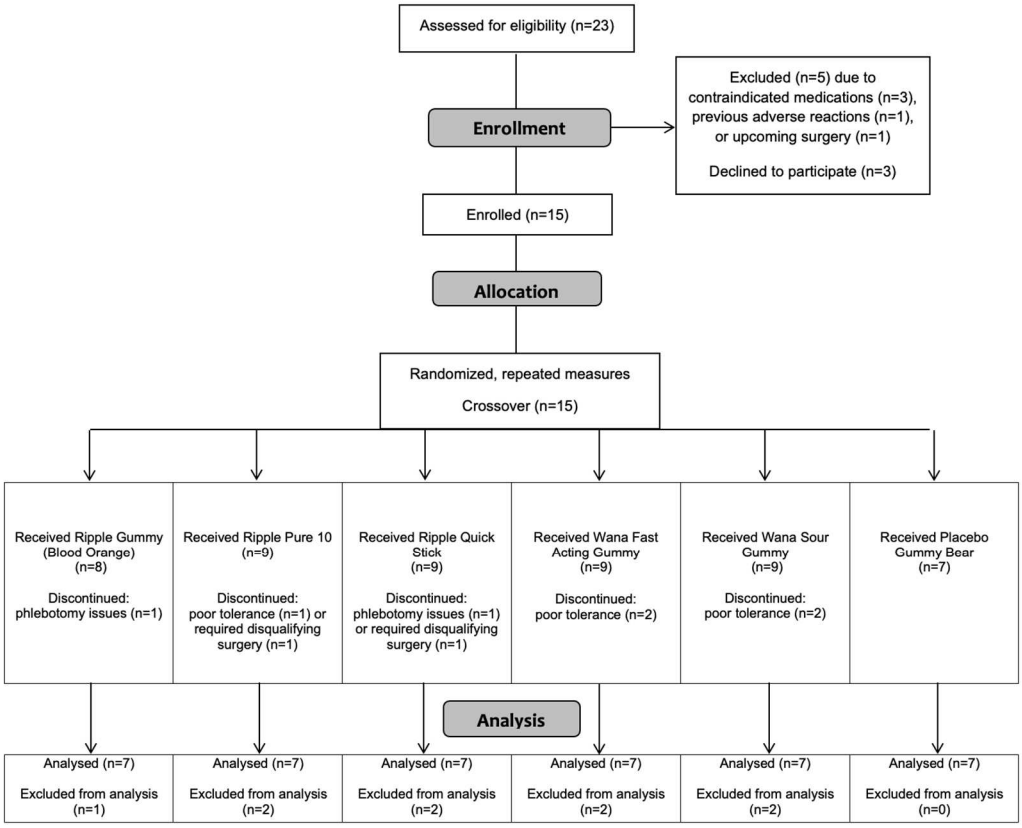

Figure 1: Consolidated Standards of Reporting Trials (CONSORT) diagram

Table 2. Selected physiological characteristics of study participants.

Characteristic	Mean ± SD	Range
Sex (M/F)	4/3	-
Age (years)	31 ± 5	24–39
Height (cm)	170 ± 11	159–193
Body Mass (kg)	82.3 ± 17.7	62.7–113.9
Body Mass Index (kg/m^2)	28.6 ± 6.5	23.0–40.8
Fat Mass (kg)	28.1 ± 13.5	16.3–55.6
Body Fat (%)	33.4 ± 10.1	21.2–48.9
Lean Mass (kg)	52.2 ± 10.0	38.1–66.0
Bone Mineral Content (kg)	2.3 ± 0.4	2.0–3.1

Figures 2, 3, and 4 depict the average circulating concentrations of THC, THC-OH, and THC-COOH for each of the five products. There was no statistical (product x time) difference between any of the products for the metabolites THC-OH (p = 0.415) or THC-COOH (p = 0.485). However, there was a difference between products for mean circulating THC concentrations (p = 0.019). Post-Hoc Tukey test analysis revealed that at minute twenty, Ripple Blood Orange Gummies led to a significantly higher plasma THC concentration than Wana Fast Acting Gummies (p = 0.003), and Wana Sour Gummies (p < 0.001). Additionally, at minute thirty, both Ripple Blood Orange Gummies and Ripple Pure 10 products led to a significantly higher plasma THC concentration than Wana Sour Gummies (p = 0.002 and 0.038 respectively).

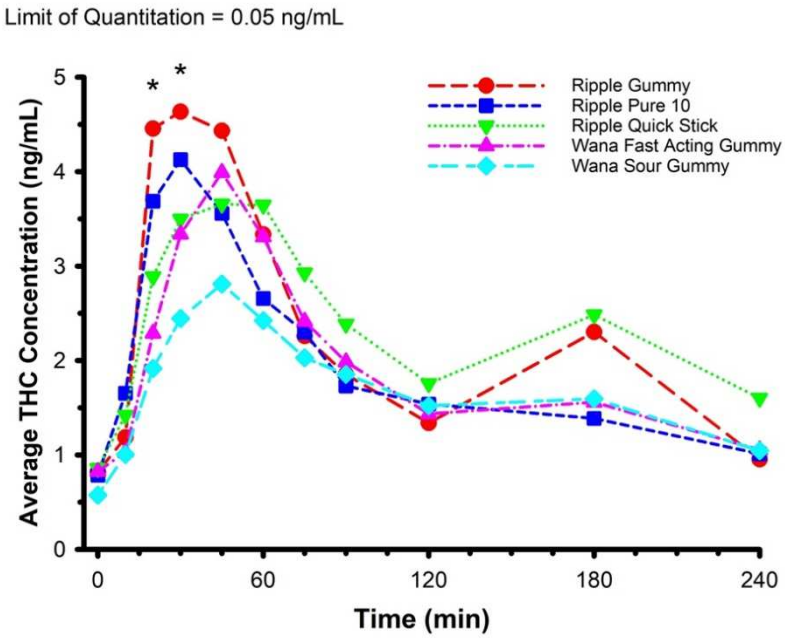

Figure 2. Mean circulating concentrations of THC following ingestion of commercially available edible marijuana (10 mg of THC). * Represents product × time interaction ($p = 0.019$). Post hoc analysis revealed circulating THC concentration was greater in Ripple Blood Orange Gummies vs. Wana Fast Acting Gummies ($p = 0.003$) and Wana Sour Gummies ($p < 0.001$) at 20 min, and greater in Ripple Blood Orange Gummies vs. Wana Sour Gummies ($p = 0.002$), and Ripple Pure 10 vs. Wana Sour Gummies ($p = 0.038$) at 30 min. Error bars have been omitted for clarity.

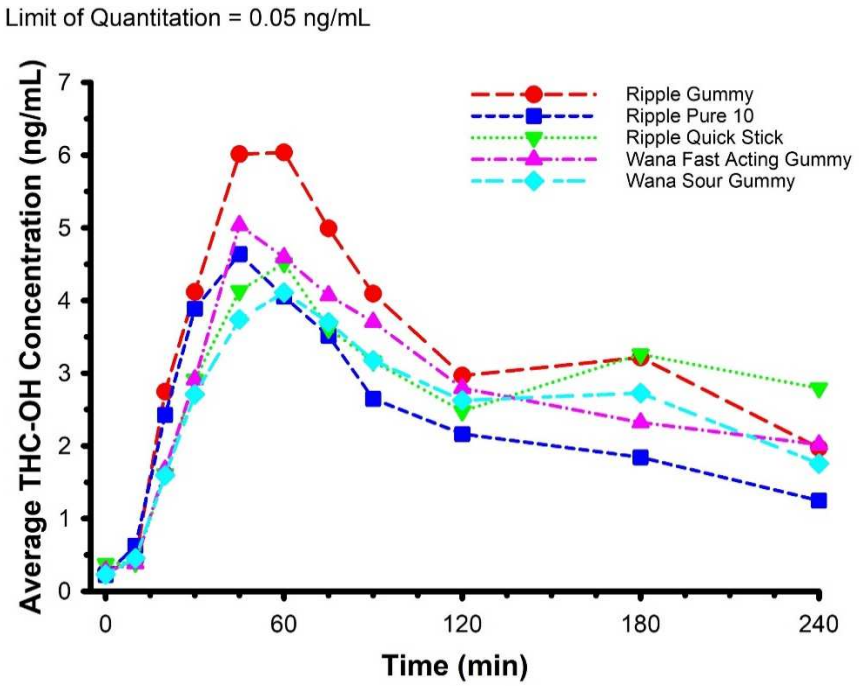

Figure 3. Mean circulating concentrations of THC-OH following ingestion of commercially available edible marijuana (10 mg of THC). There were no product × time interactions ($p = 0.415$). Error bars have been omitted for clarity.

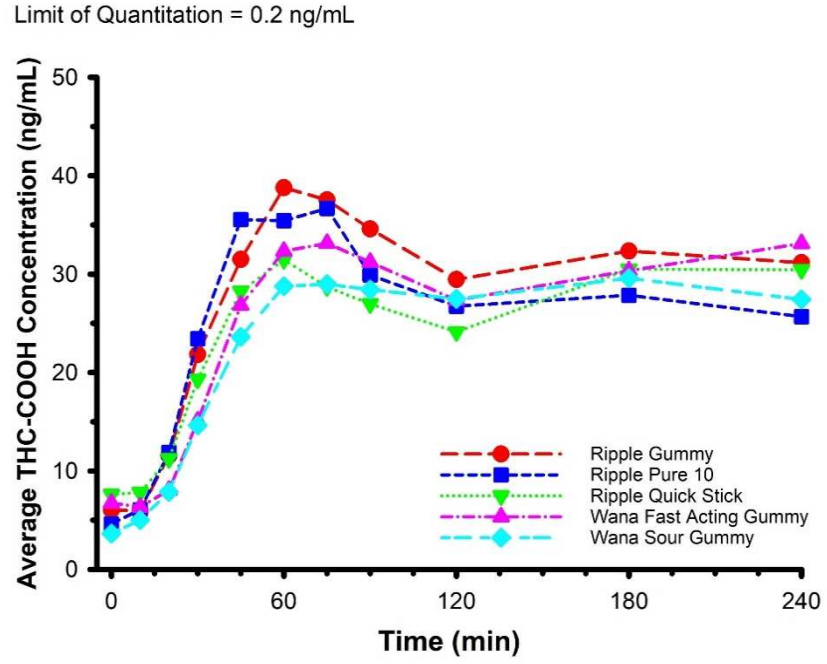

Figure 4. Mean circulating concentrations of THC-COOH following ingestion of commercially available edible marijuana (10 mg of THC). There were no product × time interactions ($p = 0.485$). Error bars have been omitted for clarity.

Tables 3-5 show pharmacokinetic data from time 0-240 (minutes). There were no significant differences in any of the parameters for THC (table 3), THC-OH (table 4), and THC-COOH (table 5) (all $p > 0.06$).

Table 3. Pharmacokinetic parameters for THC.

Product		T_{Max} **(min)**	C_{Max} **(ng/mL)**	**AUC_{0-240} (min * ng/mL)**	**V_d (mL)**	**CL/F_{0-240} (mL/min)**	**k_e (L/min)**	**$t_{1/2}$ (min)**
Ripple Gummies	n	7	7	7	7	7	7	7
	$\bar{x}$	35.7	5.54	533	4,534,900	19,313	0.005	268.3
	σ	12.1	3.10	286	4,406,250	20,443	0.003	267.0
	$\bar{\mu}$	45.0	5.22	463	2,979,599	14,131	0.00	152.7
Ripple Pure 10	n	7	7	7	6	6	6	6
	$\bar{x}$	40.7	4.31	447	4,397,542	23,966	0.005	152.4
	σ	11.3	3.01	301	2,282,851	17,028	0.002	47.1
	$\bar{\mu}$	45.0	2.37	271	4,531,448	22,193	0.000	148.6
Ripple Quick Sticks	n	7	7	7	5	5	5	5
	$\bar{x}$	90.7	4.56	570	2,648,627	11,844	0.006	215.5
	σ	84.6	1.80	268	1,272,398	5691	0.004	175.0
	$\bar{\mu}$	60.0	5.17	632	2,369,956	12,915	0.000	206.3
Wana Fast Acting Gummies	n	7	7	7	6	6	6	6
	$\bar{x}$	51.4	4.39	455	4,989,024	19,431	0.005	158.8
	σ	31.1	2.91	248	4,898,405	14,590	0.002	75.5
	$\bar{\mu}$	45.0	4.29	421	2,401,279	14,491	0.010	133.5

	n	7	7	7	6	6	6	6
Wana Sour Gummies	$\bar{x}$	62.1	3.22	406	3,960,415	16,420	0.004	180.0
	σ	53.0	2.04	296	1,463,937	7526	0.001	68.3
	$\bar{\mu}$	45.0	2.57	305	4,462,147	15,837	0.000	165.5
	P-	0.548	0.110	0.210	0.468	0.446	0.697	0.684
	F-	3.061*	2.12	1.587	0.928	0.973	0.556	0.576

T_{Max}: the time to maximum concentration. C_{Max}: the maximum concentration. $AUC_{0\text{-}240}$: the area under the curve representing total THC exposure between time 0 and end of data collection. V_d: the volume of distribution, an estimate of the degree to which THC is distributed in the body tissue vs. the plasma. $CL/F_{0\text{-}240}$: the apparent total clearance of the THC from plasma after oral administration. K_e: the rate at which the THC is removed from the body. t½: the amount of time it takes to decrease the circulating concentration to half of its initial value. All product servings contained 10 mg of THC. $\bar{x}$ represents mean value. σ represents the standard deviation. $\bar{\mu}$ represents median value. *P*- represents statistical *p*-value. *F*- represents the *F*-value from the ANOVA table unless depicted by *, in which case this is the Chi-square value.

Table 4. Pharmacokinetic parameters for THC-OH.

Product		T_{Max} **(min)**	C_{Max} **(ng/mL)**	AUC_{0-240} **(min * ng/mL)**	V_d **(mL)**	CL/F_{0-240} **(mL/min)**	k_e **(L/min)**	$t_{1/2}$ **(min)**
Ripple Gummies	n	7	7	7	7	7	7	7
	$\bar{x}$	55.7	6.60	816	2,993,411	7138	0.005	512.8
	σ	16.7	3.42	361	3,760,404	2809	0.002	989.6
	$\bar{\mu}$	45.0	7.97	950	1,284,652	7100	0.000	149.3
Ripple Pure 10	n	7	7	7	7	7	7	7
	$\bar{x}$	53.6	5.05	560	3,628,286	17,124	0.005	159.4
	σ	17.0	4.20	359	2,117,850	13,390	0.001	49.0
	$\bar{\mu}$	45.0	3.32	447	3,804,011	11,432	0.000	145.8
Ripple Quick Sticks	n	7	7	7	5	5	5	5
	$\bar{x}$	100.7	5.33	700	3,036,162	8389	0.003	403.7
	σ	77.3	2.71	381	1,634,370	6850	0.003	335.4
	$\bar{\mu}$	60.0	4.62	747	3,076,976	8413	0.000	267.9
Wana Fast Acting Gummies	n	7	7	7	5	5	5	5
	$\bar{x}$	83.6	5.40	669	1,773,924	6703	0.005	213.1
	σ	49.6	3.71	361	783,269	2218	0.002	154.4
	$\bar{\mu}$	60.0	4.83	753	1,633,620	6014	0.000	138.8
	n	7	7	7	6	6	6	6

Wana Sour Gummies	$\bar{x}$	72.9	4.45	626	2,141,277	8860	0.005	234.0
	σ	48.6	2.25	310	956,697	5405	0.005	199.8
	$\bar{\mu}$	60.0	4.36	544	1,811,579	7708	0.000	189.1
	P-	0.369	0.390	0.065	0.758	0.169	0.975	0.778
	F-	4.283 *	1.076	2.553	0.468	1.808	0.117	0.440

T_{Max}: the time to maximum concentration. C_{Max}: the maximum concentration. $AUC_{0\text{-}240}$: the area under the curve representing total THC-OH exposure between time 0 and end of data collection. V_d: the volume of distribution, an estimate of the degree to which THC-OH is distributed in the body tissue vs. the plasma. $CL/F_{0\text{-}240}$: the apparent total clearance of the THC-OH from plasma after oral administration. K_e: the rate at which the THC-OH is removed from the body. t½: the amount of time it takes to decrease the circulating concentration to half of its initial value. All product servings contained 10 mg of THC. $\bar{x}$ represents mean value. σ represents the standard deviation**.** $\bar{\mu}$ represents median value. *P*- represents statistical *p*-value. *F*- represents the *F*-value from the ANOVA table unless depicted by *, in which case this is the Chi-square value.

Table 5. Pharmacokinetic parameters for THC-COOH.

Product		T_{Max} **(min)**	C_{Max} **(ng/mL)**	AUC_{0-240} **(min * ng/mL)**	V_d **(mL)**	CL/F_{0-240} **(mL/min)**	k_e **(L/min)**	$t_{1/2}$ **(min)**
Ripple Gummies	n	7	7	7	4	4	4	4
	$\bar{x}$	105.0	44.01	7047	266,200	484	0.002	365.3
	σ	75.0	21.32	3264	140,158	147	0.000	97.3
	$\bar{\mu}$	60.0	34.28	6251	241,269	514	0.000	325.7
Ripple Pure 10	n	7	7	7	6	6	6	6
	$\bar{x}$	87.9	40.24	6311	360,519	569	0.001	846.3
	σ	67.9	19.44	3137	194,482	420	0.001	986.7
	$\bar{\mu}$	60.0	34.15	5154	299,004	568	0.000	487.8
Ripple Quick Sticks	n	7	7	7	3	3	3	3
	$\bar{x}$	130.7	42.25	6195	288,446	802	0.003	272.2
	σ	86.0	22.51	3667	28,675	344	0.001	80.7
	$\bar{\mu}$	75.0	35.63	5870	301,043	674	0.000	309.4
Wana Fast Acting Gummies	n	7	7	7	3	3	3	3
	$\bar{x}$	145.7	39.36	6467	312,251	363	0.001	611.4
	σ	84.3	15.12	2798	201,295	247	0.000	73.8
	$\bar{\mu}$	180.0	41.04	6203	208,977	262	0.000	585.7

Product		T_{Max} (min)	C_{Max} (ng/mL)	AUC_{0-240} (min * ng/mL)	V_d (mL)	CL/F_{0-240} (mL/min)	k_e (L/min)	$t_{1/2}$ (min)
	n	7	7	7	2	2	2	2
Wana Sour Gummies	$\bar{x}$	145.7	35.78	6009	297,051	758	0.003	677.6
	σ	68.6	18.89	3324	81,970	789	0.003	780.3
	$\bar{\mu}$	180.0	29.09	5119	297,051	758	0.000	677.6
	P-	0.514	0.746	0.642	0.134	0.680	0.107	0.860
	F-	3.270 *	1.943 *	2.514 *	2.533	0.591	2.852	0.314

T_{Max}: the time to maximum concentration. C_{Max}: the maximum concentration. AUC_{0-240}: the area under the curve representing total THC-COOH exposure between time 0 and end of data collection. V_d: the volume of distribution, an estimate of the degree to which THC-COOH is distributed in the body tissue vs. the plasma. CL/F_{0-240}: the apparent total clearance of the THC-COOH from plasma after oral administration. K_e: the rate at which the THC-COOH is removed from the body. t½: the amount of time it takes to decrease the circulating concentration to half of its initial value. All product servings contained 10 mg of THC. $\bar{x}$ represents mean value. σ represents the standard deviation. $\bar{\mu}$ represents median value. *P*- represents statistical *p*-value. *F*- represents the *F*-value from the ANOVA table unless depicted by *, in which case this is the Chi-square value.

Pearson correlations between body composition and THC pharmacokinetics are presented in table 6. While some body composition parameters were significantly correlated with THC pharmacokinetics, none of these correlations remained significant across all edible products and significant correlations had widely differing R-values between pharmacokinetic parameters.

Table 6. Pearson correlations between parameters of body composition and THC pharmacokinetics.

	Age	Ht	BMC	Fat Mass	Lean Mass	Total Mass	% Fat	BMI

T_{Max}	Ripple Gummies	r	0.61	0.54	0.65	0.22	0.90	0.71	−0.24	0.42
		p	0.15	0.21	0.12	0.63	**0.01**	0.07	0.61	0.35
	Ripple Pure 10	r	−0.16	0.91	0.87	−0.63	0.66	−0.08	−0.90	−0.54
		p	0.74	**0.01**	**0.01**	0.13	0.11	0.87	**0.01**	0.21
	Ripple Quick Sticks	r	−0.62	−0.20	−0.33	0.07	−0.33	−0.12	0.21	0.00
		p	0.13	0.67	0.48	0.89	0.47	0.79	0.65	1.00
	Wana Fast Acting Gummies	r	0.50	0.01	0.03	0.09	0.33	0.13	−0.14	0.10
		p	0.25	0.98	0.95	0.85	0.47	0.79	0.76	0.83
	Wana Sour Gummies	r	−0.32	0.87	0.87	−0.35	0.58	0.09	−0.53	−0.36
		p	0.47	**0.01**	**0.01**	0.45	0.17	0.85	0.22	0.43
C_{Max}	Ripple Gummies	r	−0.24	−0.68	−0.77	0.30	−0.80	−0.25	0.58	0.10
		p	0.61	0.09	**0.04**	0.51	**0.03**	0.58	0.17	0.82
	Ripple Pure 10	r	−0.21	−0.48	−0.60	0.35	−0.62	−0.12	0.58	0.13
		p	0.65	0.28	0.15	0.44	0.14	0.80	0.17	0.79
	Ripple Quick Sticks	r	−0.21	0.03	−0.04	−0.19	−0.31	−0.34	−0.10	−0.36
		p	0.65	0.95	0.93	0.68	0.50	0.45	0.84	0.43
	Wana Fast Acting Gummies	r	−0.07	−0.36	−0.46	0.29	−0.38	−0.00	0.31	0.18
		p	0.89	0.43	0.30	0.53	0.40	0.99	0.51	0.69
	Wana Sour Gummies	r	0.21	−0.63	−0.66	0.73	−0.42	0.29	0.84	0.60
		p	0.65	0.13	0.11	0.06	0.34	0.53	**0.02**	0.16
AUC_{0-240}	Ripple Gummies	r	0.05	−0.63	−0.75	0.65	−0.49	0.20	0.74	0.52
		p	0.92	0.13	0.05	0.12	0.27	0.67	0.06	0.23
	Ripple Pure 10	r	−0.12	−0.34	−0.54	0.56	−0.32	0.23	0.59	0.40
		p	0.80	0.46	0.21	0.19	0.49	0.62	0.17	0.38
	Ripple Quick Sticks	r	−0.17	−0.06	−0.23	0.03	−0.25	−0.13	0.04	−0.10
		p	0.71	0.89	0.62	0.95	0.58	0.78	0.93	0.83
	Wana Fast Acting Gummies	r	0.07	−0.43	−0.52	0.39	−0.34	0.10	0.38	0.32
		p	0.88	0.34	0.24	0.39	0.46	0.83	0.40	0.49
	Wana Sour Gummies	r	0.35	−0.47	−0.52	0.89	−0.13	0.59	0.83	0.82
		p	0.44	0.29	0.23	**0.01**	0.79	0.16	**0.02**	**0.02**

n = 7 for all cells. Ht: Height. BMC: Bone Mineral Content. BMI: Body Mass Index. $AUC_{0\text{-}240}$: Area under the concentration curve between time 0 and 240 min (4-h).

Mean plasma glucose concentrations are shown in figure 5. Plasma glucose was not different between participants at baseline ($p = 0.4$). Additionally, there were no differences from baseline in circulating glucose concentrations following ingestion of marijuana or the marijuana free control product at minute 30 prior to consumption of the 75g glucose drink ($p = 0.88$), indicating that the carbohydrate content of the product consumed was insufficient to incur a change in circulating glucose concentration. Additionally, there were no differences in circulating glucose concentrations following consumption of the glucose drink between any of the products and the marijuana free control at any time (product x time interaction $p = 0.98$), nor was a difference identified in the 2- or 3.5-hour glucose area under the curve (2-hour $p = 0.98$; 3.5-hour $p = 0.92$).

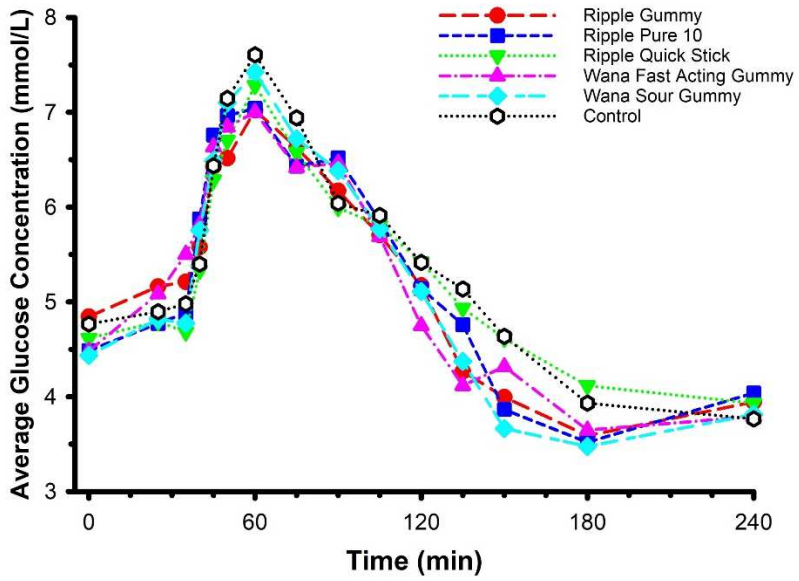

Figure 5. Mean circulating glucose concentrations following ingestion of commercially available edible marijuana (10 mg of THC) and one marijuana-free control product. Seventy-five grams of glucose was ingested at 30 min. Fasting glucose was not different across study sessions ($p = 0.40$). Circulating glucose was not different from fasting glucose 30 min after product ingestion ($p = 0.88$). Compared with placebo, none of the edible marijuana products influenced circulating glucose throughout each of the trials (product × time interaction $p = 0.98$). Error bars have been omitted for clarity.

Finally, insulin data are presented in figure 6 for the two products that invoked the highest plasma THC concentrations as well as the marijuana free control. Consistent with the data above, there were no differences in circulating insulin concentrations between products ($p = 0.74$), nor were there differences in the 2-hour insulin area under the curve ($p = 0.7$). We also found no differences in the MATSUDA index ($p = 0.4$, data not shown), nor the homeostatic model of insulin resistance (HOMA-IR; $p = 0.3$, data not shown), common indices of combined glucose and insulin concentration data.

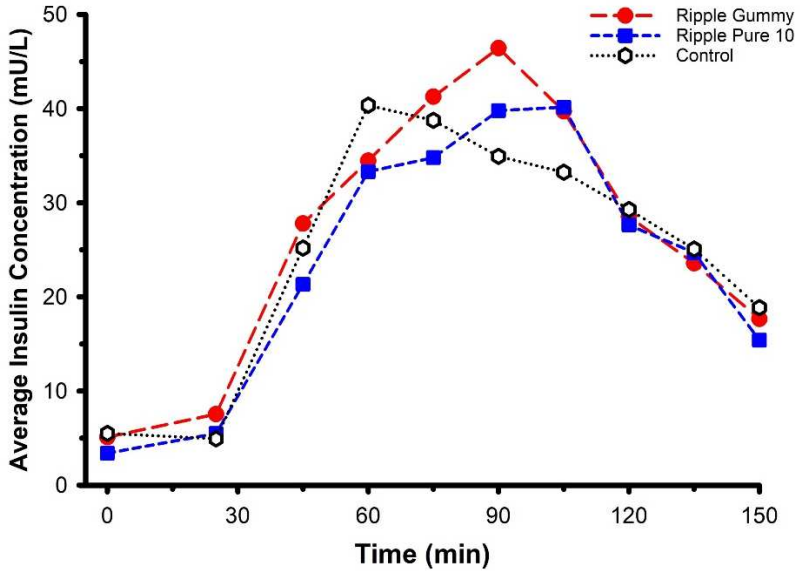

Figure 6. Mean circulating insulin concentrations following ingestion of two commercially available edible marijuana (10 mg of THC) and one marijuana-free control product. Seventy-five grams of glucose was ingested at 30-min. There were no differences in circulating insulin between products (product × time interaction $p = 0.74$). Error bars have been omitted for clarity.

DISCUSSION

We investigated three hypotheses: 1. There would be no difference in THC pharmacokinetics between varying formulations (although all edible) standardized to a 10mg dose, 2. Body composition would correlate with pharmacokinetic parameters related to THC, and 3. Marijuana ingestion would lead to favorable modification of glycemic control when compared to a marijuana-free control product. Related to the first hypothesis, we discovered that two of the formulations (Ripple Blood Orange Gummies and Ripple Pure 10) evoked plasma THC concentrations at minutes 20 and 30 that were greater than Wana Sour Gummies and Wana Fast Acting gummies, respectively. For hypothesis two, we found that body composition may influence some pharmacokinetic parameters related to THC. Our data did not support our final hypothesis. Acute THC ingestion did not influence any outcomes related to oral glucose tolerance.

There exists considerable variability in pharmacokinetic parameters among edible marijuana products. Average T_{Max} for the products we investigated ranged from 35-90 minutes, and the average C_{Max} ranged from 2.37 to 5.22 ng/mL. On the other hand, the FDA approved synthetic THC medication Dronabinol, has a T_{Max} of 1-1.5 hours and a C_{Max} of 1.81-2.2ng/mL when administered as a 4.25mg solution or a 5mg capsule[56]. Even greater variability exists when edible marijuana is integrated into other food, such as brownies. For example, in a previous study of marijuana brownies containing 20mg of THC, T_{Max} ranged from 1-to-2 hours, and C_{Max} from 2.5-to-4ng/mL[54]. Despite four times as much THC, the 20mg brownie had similar T_{Max} to

Dronabinol and a slower T_{Max}, and lower C_{Max}, compared to some of the products we investigated. There are several possible explanations to account for these differences. First, pertaining to the edible brownie data, both our products and Dronabinol are specifically engineered to deliver THC, whereas such considerations may not be prevalent in casual baking. Additionally, the brownies consist of various macronutrients, including fat, which may influence gastric emptying time and delay the time from ingestion to intestinal absorption, but may also increase total bioavailability due to THC's lipophilic nature. In other words, consuming edible marijuana with food may decrease T_{Max} due to nutrient modification of gastric emptying time, but increase C_{Max} due to the synergistic intestinal co-transport of THC with lipid. Inter-individual variability can also influence THC pharmacokinetic parameters. For example, genotype may play a role[57]. Genetic variations in the cannabinoid receptor 1 (CB1) can influence the binding of THC to the CB1 receptor and thus subsequently modify plasma THC concentrations. A heterogeneous study population based on body composition (discussed below) or genetic profile may explain the considerable pharmacokinetic variability between edible marijuana products.

Body composition may influence pharmacokinetic values for THC and its metabolites. THC is a lipophilic molecule that can be stored in adipose tissue. During periods of lipolysis, such as weight loss, fasting, or exercise, THC can be found in the plasma of individuals with previous THC exposure[11]. Additionally, fat biopsies taken from heavy users four weeks post-cessation contain quantifiable amounts of THC[58]. Thus, individuals with greater amounts of fat mass may absorb THC from plasma into lipid more readily than a leaner individual, causing lower plasma THC readings. Beyond fat mass, other body composition parameters, such as lean mass, may influence THC pharmacokinetics. For example, blood volume is positively related to lean

mass[59], so an individual with a high amount of lean mass (often acquired through regular resistance training) may have a greater blood volume in which to dilute THC, returning test values that indicate a lower circulating concentration. However, despite the proposed mechanistic links between body composition and THC pharmacokinetics, our data did not indicate that any of our body composition parameters were consistently relevant in predicting plasma THC concentration. There were several significant correlations, but none persisted across all formulations or appeared otherwise noteworthy as a physiologically relevant predictor of THC bioavailability. The heterogenous nature of our participants (e.g., BMI values ranging from 23-41 kg/m^2) might have made a significant correlation easy to detect, but it is also possible that our low participant count may have provided insufficient statistical power to detect a meaningful correlation. Finally, despite our attempts at formulating a rationale, it is also possible that body composition and THC pharmacokinetic parameters are unrelated.

Recent epidemiological research suggests that regular THC ingestion may help improve blood glucose regulation. However, to the best of our knowledge, only three studies have evaluated the potential impact of acute THC consumption on glycemic control. These studies were conducted several decades ago, in the 1970s. Two of the studies had their participants inhale combusted marijuana and had differing conclusions – one reported diminished glucose tolerance with THC inhalation while found the other no effect[49,50]. The final study used intravenous THC administration and reported diminished glucose tolerance[60]. These data directly conflict with the much more recent epidemiological studies mentioned above and may not reflect marijuana usage in the 21st century (e.g. intravenous administration is neither common nor practical). Additionally, both agricultural techniques and marijuana synthesis/manufacturing techniques are

more advanced than they were fifty years ago, leading to a potentially more potent product. Considering the above, a strength of our study was the utilization of commercially available edible marijuana products. Regardless, we found no influence of these edible marijuana products on glucose control. There are a couple of plausible reasons. First, there was heterogeneity between participants regarding body composition, which by itself may make differences more difficult to detect due the influence of body composition on glucose control[61]. Additionally, our participants were generally healthy. Thus, there may have been a ceiling effect, wherein individuals with healthy glucose homeostasis have no room for improvement. Second, we investigated an acute dose of edible marijuana, whereas the epidemiological studies considered chronic usage. This is important because our participants may already be reaping the rewards of chronic regular marijuana use (>4x month) and there was likely no novel stimulus from an acute dose in their glucose regulation. Future studies may consider using a similar field observational approach in adults with diabetes or requiring habitual THC users to abstain for a period of time (i.e. a THC washout period) to simulate the potential influence of an acute THC dose on simulated naïve users.

There are a couple of limitations worth discussing. First is our low number of participants who completed the study. Figure 1 (CONSORT diagram) shows we enrolled 15 participants in our study but only 7 completed the entire protocol. Five of the participants withdrew due to an intolerance of the study protocol or an inability to abstain from marijuana for the required 96-hour period prior to each visit. Such a withdrawal rate may indirectly support arguments that marijuana is, in fact, addictive[62], despite lay-press articles to the contrary. Additionally, the participant burden imposed by six weeks consisting of 96-hour sobriety periods may have caused

this attrition. Future research may consider using fewer study visits as a strategy to increase participant retention. An alternative explanation is that the participants may have been unaccustomed to co-ingesting marijuana and 75g glucose while in a fasted state We chose to have our participants fast to prevent any influence of post-prandial metabolism on our pharmacokinetic and glucose tolerance data, future research should weigh these concerns in accordance with the research question. In any case, scientists should factor in the notable rate of attrition when planning future research and increase recruitment accordingly to provide the desired level of statistical power. Nevertheless, considering the dearth of research on the pharmacokinetics of commercially available edible marijuana, we believe our data are quite useful for scientists and practitioners. Second, the hyperinsulinemic-euglycemic clamp is considered the gold standard for research into glucose control and insulin sensitivity. However, the oral glucose tolerance test has good validity and several potential advantages compared to the hyperinsulinemic-euglycemic clamp. First, the oral glucose tolerance test has good ecological validity and fits well with our design using commercially available edible marijuana products. Second, the hyperinsulinemic-euglycemic clamp involves an intravenous administration of glucose and insulin, which is not reflective of the normal digestion, intestinal transport, or first-pass metabolism involved in macronutrient metabolism. Third, the oral glucose tolerance test is easily replicable in future field/observational studies, whereas the hyperinsulinemic-euglycemic clamp is not due to extensive preparation and clinical supplies not easily taken to the field environment. The utility of field-practical replicability will be necessary until federal marijuana legislation changes. A final consideration for this study is the use of edible marijuana with 0% CBD content. Recent evidence has suggested that combined THC and CBD consumption may provide a synergistic “entourage effect” wherein combined consumption of both molecules may

influence the pharmacokinetics values for either[63]. Future studies should consider the composition of the product their participants are taking when evaluating pharmacokinetic data.

To summarize, differences within edible marijuana products may be evident even in products specifically engineered for maximal bioavailability, which may be relevant in marijuana overdose prevention. Body composition may influence the pharmacokinetics of acute marijuana ingestion, although future studies with greater enrollment will be needed to validate this claim. Finally, acute edible marijuana use neither impairs nor augments glucose tolerance and the effects suggested by epidemiological research may be likely due to chronic rather than acute consumption.

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