William Harvey Research Institute

# The Effects of Diet and Regular Mixers on Alcohol Pharmacokinetics

**Supervisor:** Professor Atholl Johnston

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**Name:** Asena AVCI

**ID:** 160546923

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## ABBREVIATIONS

| |
|---|
| **ABV** Alcohol by volume |
| **ADH** Alcohol Dehydrogenase |
| **ALDH** Aldehyde Dehydrogenase |
| **AUC** Area Under Curve |
| **BMI** Body Mass Index |
| **BrAC** Breath Alcohol Concentration |
| **$C_2H_5OH$** Ethanol |
| **$CH_3CHO$** Acetaldehyde |
| **$CH_3COOH$** Acetic acid |
| **CNS** Central Nervous System |
| **$CO_2$** Carbon dioxide |
| **CoA** Coenzyme A |
| **CPDR** Cumulative percent dose recovery |
| **CYP2E1** Cytochrome P450 2E1 |
| **DOB** Date of Birth |
| **GE** Gastric Emptying |
| **$H_2O$** Water |
| **IRIS** Isotope Ratio Infrared Spectrometer |
| **MD** The Doctor of Medicine |
| **NAD** Nicotinamide adenine dinucleotide |
| **$NAD^+$** reduced form of nicotinamide adenine nucleotide |
| **$NADH_2$** oxidized form of nicotinamide adenine nucleotide |
| **NDIRS** Non-Dispersive Infrared Spectroscopy |
| **NHS** National Health Service |
| **PDB** Pee Dee Belemnite |
| **PDR** Percent dose recovery |
| **RAS** Reticular Activating System |
| **SD** Standard Deviation |
| **TBW** Total Body Water |
| **VAS** Visual Analogue Scale |

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## ABSTRACT

**Background:** Consumption of alcoholic drinks with mixer is increasing in 'diet' varieties. These could possibly empty more quickly from the stomach and thus rise the rate of alcohol absorption while 'regular' ones including sugar has reverse effect.

**Methods:** In this study, 7 healthy volunteers were studied twice in randomized order. On each session, they drunk a vodka drink (37.5 % ABV), prepared with either 'regular' beverage including sugar (138.6 kcal), or 'diet' beverage including artificial sweetener (1.32 kcal). Their breath alcohol concentrations were taken every 15 minutes during 3 hours with lion alcolmeter® SD-400 and lion alcolmeter® 500. Simultaneously, their breath samples for gastric emptying measurement were taken in breath bags in the same time interval. These breath samples were analyzed with an IRIS® instrument to get $t_{1/2}$ and $t_{lag}$ parameters.

**Results:** Both the peak ethanol breath ethanol concentration (440±72.3 vs. 386±62.9 μg/L) and the area under the breath ethanol curve between 0 and 180 minutes (507.4±67.6 vs. 448.1±50.8 units) were greater with diet coke. Gastric half-emptying time and lag phase time were calculated from the $^{13}C$ percent dose recovery values measured by IRIS® instrument with nonlinear regression analysis. Gastric half-emptying time was less for the diet drink than the regular drink (86.3±7.8 vs. 88.4±17.1 min) but $t_{lag}$ was greater for diet drink than the regular drink (48.1±9.6 vs. 46.1±15.7 min).

**Conclusions:** Consumption of artificial sweeteners for sucrose by mixing them with alcoholic drinks may have an effect on the rate of gastric emptying and the breath alcohol response.

# 1. INTRODUCTION

## 1.1. Alcohol

Alcohol is the most utilized inebriating substance in general public. Consider these statistics (NHS Digital, 2017):

- 57 % of adults stated drinking alcohol in 2016 which is a decrease from 64 % in 2006. This means that of 25.3 million adults in England.
- Those who drank more than 8/6 units on their heaviest day dropped from 19% to 15%.
- In 2015, there were 6,813 deaths which were connected to the drinking of alcohol. This is 1.4% of all deaths.

Alcohols are hydroxy derivatives of straight or branched chain aliphatic hydrocarbons. While common alcohols may contain three hydroxyl groups with at least one on each carbon atom, less common ones may include more than one hydroxyl group per carbon atom. Latter type of alcohols involves ethanol, methanol and isopropanol and they cause toxicity most. Generally, the alcohol has a greater toxicity if the carbon chain of the alcohol is longer. However, there is an exception to this: methanol has greater toxicity than ethanol (Gossel and Bricker, 1990).

Ethanol ($C_2H_5OH$) is the alcohol obtained by the fermentation of glucose and other sugars and starches with the help of yeast activity. As ethanol is the most significant alcohol for social and economic aspects, the word ‘alcohol’ generally refers to ethanol. Ethanol is the only most essential drug in forensics because it is ingested in a widespread manner, it has detrimental consequences and there is a law with respect to drinking and driving (Ferner and Norman, 1996). Ethanol has clear, colourless characteristics and it reveals a sense of burning in the mouth and throat when it is consumed. It is an influential central nervous system (CNS) sedative that effects mainly the reticular activating system (RAS). Moreover, it acts nearly the same as general anaesthetics (Gossel and Bricker, 1990).

There can be three categories of alcoholic drinks: alcohols produced by just fermenting, such as beers and table wines; alcohols whose concentration has been raised by distilling, such as spirits and liquors; fortified wines (e.g. port, sherry and vermouth) whose concentration is risen by original fermenting level with distilled spirit (e.g. brandy). While drinking an alcoholic beverage, there is an unawareness of taking a drug. On the consumption of alcohol, people will be less aware of the feeling of depression or tiredness and when they consume alcohol at reasonable levels, they will lose inhibition and self-restraint. Higher alcohol levels cause damage to consciousness and death related to respiratory paralysis because of acute alcohol intoxication (Emerson, 2004).

### 1.2.Carbonated Drinks

Currently, the carbonated beverages industry is one of the largest income making businesses around world. People are drinking great quantities of carbonated beverages to satisfy their dehydration. There are many products in the marketplace and Coca-Cola is one of them.

#### 1.2.1. Regular Coke vs. Diet Coke

Regular and Diet Coke have the identical constituents except for the kind of sweeteners utilised in their manufacture. This difference happened because of the demand for a soft drink that is less fatty and which can be used by diabetic patients.

Regular Coke is sweetened with sugar produced from high fructose corn syrup which is high in calories and can cause a spike in insulin amount in the body. Conversely, Diet Coke is sweetened with synthetic sweeteners like aspartame which is an artificial sweetener having lesser calories (Emelda, 2011).

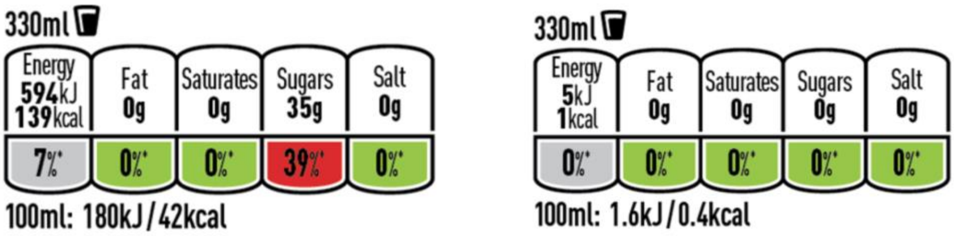



**Figure 1** Nutrition information for a can of Regular Coke vs. Diet Coke (The Coca-Cola Company, 2017).

The taste of regular coke is very different and fulfilling. However, a can of regular coke includes nearly 138 calories, thus; consumption of it would results in weight gain. Also, it has a sweeter taste because of its sugar constituent. On the other hand, the taste of Diet Coke is a little flavourless at first because it does not have some of the ingredients of the original coke. It is chosen by people paying attention to their sugar consumption and their weight because it has less calories and no sugar (Emelda, 2011).

### 1.2.2. History of Diet Coke and Regular Coke

Coca-Cola Classic is the world's most common soft drink and has been appreciated since 1886. It is created by Dr John Pemberton and it first was put up for sale at Jacob's Pharmacy in Atlanta, Georgia. Actually, it was Dr Pemberton's accountant, Frank Mason Robinson, who designed the current world renowned red-and-white logo with its unique Spencerian pattern. Coca-Cola Classic appeared in Great Britain in 1900, when Charles Candler brought a bottle of syrup with him on a trip to England. After that, in the beginning of 1920s, it was put on the market in Selfridges and at the London Coliseum. Ingredients for Coca Cola Classic: Carbonated Water, Sugar, Colour (Caramel E150d), Phosphoric Acid, Natural Flavourings Including Caffeine.

Diet Coke was released in the US in 1982 and came Great Britain in the following year. It is well-known for its impressive marketing campaigns and associates with style designers. Ingredients for Diet Coke: Carbonated Water, Colour (Caramel E150d), Sweeteners (Aspartame, Acesulfame K), Natural Flavourings Including Caffeine, Phosphoric Acid, Citric Acid. Contains a Source of Phenylalanine. (The Coca-Cola Company, 2017).

### 1.3.Effect of Diet and Regular Mixers on Alcohol

Alcohol includes calories and people often consume alcohol with diet beverages to prevent added calories coming from regular beverages (Marczinski, 2013). Diet Coke contains no calories, but the added ingredient aspartame complicates Diet Coke as a healthy alternative in order to lose weight and eat healthier. Aspartame is metabolised by the body into three constituents: methanol, phenylalanine and aspartic acid. Each of these substances can have disturbing effects on the body. Methanol is altered to formaldehyde by the body. Known for its use as an embalming fluid, formaldehyde is not something that many people voluntarily put into their bodies. It has been proven that even low levels of methanol and formaldehyde are toxic. Phenylalanine is an amino acid. It is generally considered safe for everyone except for sufferers of phenylketonuria, a somewhat rare genetic disease. But it has been argued that high levels of the substance could cause neurological damage. Aspartic acid is known to be an excitotoxin. A type of neurotoxin, excitotoxins have been known to damage brain and nerve cells. This can potentially cause a number of chronic diseases (Burning Tree Rehab., 2013). Nevertheless, the European Commission Scientific

Committee on Food reviewed subsequent safety studies and reaffirmed the approval of aspartame in 2002.

In an article in Iowa State Daily, Dr. Bryan Graveline, M.D., gastroenterologist at McFarland Clinic, explained the increase in gastric emptying and alcohol absorption. After the stomach, drink passes to the next organ, the small intestine, which is where nutrients are metabolised into molecules. After that, they travel across the wall into the circulation and it is in the body. When you drink alcohol, it goes into your stomach; absorption of alcohol from the stomach is slow. But once in the small intestine, alcohol is rapidly absorbed into the bloodstream and goes to the brain and the rest of the body. The study showed regular beverages with alcohol take more time to empty the stomach than diet beverages. This causes less intoxication (Wu, 2006). The stomach contains alcohol dehydrogenase that transform alcohol into acetaldehyde, which does not result in contribute to intoxication. When you are drinking diet mixers, the stomach takes less time for emptying. The alcohol leaves the stomach and passes to small intestine, and it does not have time to be decomposed. Basically, if the stomach empties faster, then the alcohol moves into the small intestine and more alcohol is moved into the blood stream (McGrath, 2013).

## 2. PHARMACOKINETICS OF ALCOHOL

When investigating drink-driving and sudden death cases, a forensic scientist is needed for the determination of the amount of alcohol consumed. For the accomplishment of this investigation, absorption, distribution in the body and elimination of alcohol have to be understood by the forensic scientist (Emerson, 2004).

### 2.1. Absorption

When alcohol is consumed, it moves from mouth across the mucosa which is the mucous membrane layer of the stomach which includes the glands and the gastric pits. Then, it goes into the blood which travel in the vessels that provide it, by the time that the blood ethanol concentration is equal to the ethanol concentration in the lumen of the stomach (Ferner and Norman, 1996).

Ethanol has some physicochemical characteristics such as slight polarization and weak electrical charge and low molecular mass. It has miscibility in water and solubility in lipids. Consequently, absorption of alcohol is easy and alcohol passage through cell membranes can occur by simple diffusion (Gossel and Bricker, 1990). Simple diffusion is a process that alcohol does not undergo any chemical change and it follows Fick's law which explains the factors affecting the rate of diffusion. These factors are the concentration of the alcohol, local blood stream and the type of the absorbing surface. Diffusion of only nearly 20% of the consumed alcohol by the gastric mucosa occurs due to the fact that the stomach has relatively small surface area and its retention time is comparatively short. Then, the alcohol moves through the small intestine that absorbs the remaining 80% of alcohol (Emerson, 2004).

Ethanol absorption from the stomach after it is taken from the mouth initiates on ingestion. It is slightly absorbed till its entrance to the stomach because the passage of liquids from the mouth through the gullet of the stomach is fast. After a while, stomach emptying occurs, its constituents pass to the small intestine. In the small intestine which has much larger surface area, the absorption of ethanol continues. The small intestine has thinner mucosal lining than the stomach and the blood supply of the small intestine has rich blood supply. Therefore, the ethanol diffuses more quickly through the mucosa of the small intestine than through the mucosa of the stomach. Consequently, the rapidity of the ethanol reaching the small intestine affects the overall rate of the absorption. Moreover, the absorption of ethanol by the colon and the large intestine can occur if the ethanol is taken by an enema (Ferner and Norman, 1996).

The absorption of alcohol on an empty stomach is fast. In one study, they investigated the rate and amount of absorption when eight normal subjects took different doses of alcohol. Subjects are analysed four times and they took doses of 11.2 grams (9.4% by volume), 22.4 grams (18.8%), 33,6 grams (28.2%) and 45.0 grams (37.9%) of ethanol represented 150 millilitres with orange juice. Then, blood samples were drawn and the interpretation of the results by a mathematical model was made. This mathematical model explained gastric emptying and absorption as a first order process. Blood peak concentrations are obtained at 22.5 min, 40 min, 55 min and 60 min, which are the average times, after consuming 11.2, 22.4, 33.6 and 45 grams of ethanol in a respective manner. After some assumptions and approximations, the rate constant for the absorption of ethanol from the bowel was found to be 25.0 $h^{-1}$. Its half-life is around 1.66 minutes. In other words, the absorption of more than 98% of alcohol in the small intestine could be occurred in 10 minutes. In this model, peak absorption is reached with a delay which is because of the relative retardation of gastric emptying. Practically, the consumption of ethanol is happened more or less consistently in a period of time, and the absorption rate is a significant element for the determination of the rapidity of reaching the peak ethanol concentration (Wilkinson, cited in Ferner and Norman, 1996). In addition to the rate of gastric emptying which is also related to the volume and nature of stomach contents, there are various factors that affect the rate of absorption. Stomach volume and type of its contents affect the function of pyloric sphincter which is the muscular valve managing the emptying of components from the stomach. When large amount of beer is consumed, the stomach distends and it causes that pyloric sphincter undergoes muscle spasm because of ingestion of ethanol with the concentration over 30% by volume such as in neat alcoholic drinks. If there is food in the stomach, the rate of stomach emptying is decreased due to the dilution of the ethanol entering the stomach and the limitation of the interaction between the ethanol and the gastric mucosa. Due to all these reasons, ethanol is absorbed more quickly and more completely in an empty stomach. Furthermore, medicinal drugs may influence the gastric emptying rate and thus ethanol absorption rate. Some drugs slow gastric emptying such as atropine, chlorpromazine and promazine, disopyramide, procyclidine, propantheline while some drugs such as the antiemetics metoclopramide and cisapride and the antibiotic erythromycin accelerates stomach emptying. Moreover, partial gastrectomy, which is the operation for the removal of some part of the stomach because of peptic ulcer disease treatment, fastens gastric emptying (Ferner and Norman, 1996).

#### 2.1.1. Factors affecting alcohol absorption

- Because of passage between biological membranes by passive diffusion, its concentration gradient decreases. Thus, when the alcohol concentration is high, its concentration gradient is also high and it results in faster absorption.
- If alcohol is removed from absorption site quickly by an efficient blood stream, the concentration gradient can be maintained and absorption can be promoted.

- Due to irritant characteristics of alcohol, high alcohol concentrations can result in surface corrosions, hemorrhages and paralysis of the stomach involuntary muscle. Consequently, alcohol absorption decreases.
- When alcohol is consumed as a single dose, maximum blood alcohol levels are greater than that in consumption with many smaller doses due to higher concentration gradient in the former.
- Generally, there is no significant effect of the kind of alcoholic drink on blood alcohol concentration.
- If there is food in the stomach, it slows gastric emptying and therefore decrease alcohol absorption. Fatty, carbohydrate or protein rich meals effectively slow down gastric emptying (Cederbaum, 2012).

## 2.2. Distribution

After absorption process, alcohol is homogeneously distributed through all tissues and body fluids. The water content and blood supply of each organ affect the distribution. There is an equilibrium condition between alcohol in the blood and tissue sections. Alcohol is distributed between alveolar air and blood in accordance with the speed of diffusion, its vapor pressure and concentration of alcohol in the lung vessels. The distribution ratio between alveolar air and blood is 1:2,100. This ratio is used when determining the blood alcohol concentrations from breath analysis methods (Gossel and Bricker, 1990).

Total body water (TBW) is the term that describes total amount of fluid in the body and it is related to alcohol distribution. It represents 60-70% of body weight, yet it changes in males and females and also affected by size. For men, it reduces with age:

$$Total\ body\ water\ (TBW) = 2.447 - 0.09516\ \times\ age +\ 0.01074 + 0.3362\ \times\ weight$$

For females, it does not depend on age:

$$Total\ body\ water\ (TBW) =\ -2.097 + 0.1069\ \times\ height + 0.2455\ \times\ weight$$

where water in litres, age in years, height in centimetres and weight in kilograms.

Women have smaller TBW because they have more fat tissue than men of identical weight. Therefore, blood alcohol concentration in men is slightly lower than after the consumption of same amount of alcohol (McGovern et. al, 2011)

Watson et. al studied on over 700 people aged between 17-86 and developed practical equations. If the subject's total body water and the blood ethanol concentration are known, ethanol in the body at a time can be determined quantitatively. The blood has a water content and this part includes ethanol not the fatty part. Therefore, the blood ethanol concentration is needed to be adjusted. The determination of the correction can be made by dehydrating a sample of blood. The assumption that blood has 80% water is made (Watson, cited in Ferner and Norman, 1996). Consequently, the ethanol concentration in the aqueous part of blood is 1.25 times the whole blood concentration:

$$\text{concentration in water} = \frac{\text{concentration in blood}}{\text{watery fraction of blood}}$$

Thus, the quantity of ethanol in the body is equal to:

$$\text{ethanol (mg)} = \frac{\text{Total body water (deciliters)} \times \text{blood ethanol concentration}\left(\frac{\text{miligrams}}{\text{deciliters}}\right)}{\text{watery fraction of blood}}$$

The volume of distribution is generally stated as the fraction of the amount of a substance in one unit volume of blood to the overall amount in the body, so TBW/watery fraction of blood. Forensic interpretation of ethanol mostly comes from the scientific research made in 1932 by Widmark. In this research, the amount of ethanol in the body was described with respect to the concentration in blood, $C_0$, the weight of the body in kilograms, p, and a factor 'r', which accounted for the volume of distribution per kilogram body weight. Widmark's equation is:

$$A = r\ x\ p\ x\ \mathrm{C0}$$

The average r values are experimentally found as 0.68 for men and 0.55 for women. With direct experiment, these values were found as 54% and 44% respectively and they are lower. Calculation of TBW with the height and weight and age in men and the correction of the blood ethanol concentration to the amount in the watery part of blood is advantageous because more information is used with this method than Widmark's method depend on sex and weight alone (Ferner and Norman, 1996).

### 2.3.Elimination

First-order elimination kinetics is used for the most of the drugs. In other words, the elimination of them from the body with a rate which is based on the quantity of drug present in the body, and the quantity of drug remaining at any certain time in the body shows an exponential decrease to zero. For alcohol, elimination occurs regarding to first order kinetics when blood alcohol concentration values are less than 20 mg/dL which is very small and clinically unimportant (Gossel and Bricker, 1990).

The conversion of ethanol into acetaldehyde with alcohol dehydrogenase is achieved by the human body. After this conversion, acetic acid and acetaldehyde are produced. Alcohol generates some clinical side effects due to acetaldehyde.

Elimination of ethanol occurs at a constant rate in each person. The average rate of decline in blood alcohol concentration is usually accepted as approximately 15 mg% per hour, however; the real range is 10-20 mg% per hour despite the fact that the limits are rarely attained excluding in people with chronic alcoholism (McGovern et. al, 2011).

Ethanol elimination is analysed quantitatively by using two approaches. A basic zero order model can be used and in this approach, the rate of elimination of ethanol is assumed to be constant. Other method depends on concentration and has limited capacity (Michaelis-Menten model). The disappearance of ethanol is determined with regards to the maximum velocity, $V_{max}$ and the half-saturated enzyme concentration, $K_m$.

Widmark determined the amount of total ethanol which is absorbed in a certain period of time, t, with the equation:

$$C_t = C_0 - \beta.t$$

where $C_t$ is the BAC at a time, t, $C_0$ the ethanol concentration at time zero, 0, and $\beta$ a constant value indicating the average quantity of ethanol eliminated in unit time. The unit of $\beta$ was milligrams ethanol per gram of blood per minute or per hour. Lately, it has been indicated as milligrams ethanol per 100 millilitres of blood per hour (Ferner and Norman, 1996).

### 2.3.1. Factors changing the elimination rate of alcohol

- **Sex:** Women eliminates alcohol with a more rapid rate when lean body masses are applied for rates. Due to lesser body size and thus lesser lean body mass, women have greater ethanol elimination per unit lean body mass (lbm). Alcohol elimination rates are similar for women and men when rate is stated as grams/hour or grams/liter liver volume.

- **Age:** Metabolism of alcohol for older people may be slower than young people. Volume of total body water reduces due to a decrease in lean body mass and an increase in adipose tissue with increasing age. Consequently, blood alcohol level of an older individual may be higher than that of a younger individual that has same body weight and consume same amount of alcohol (Dufour et al., 1992). Moreover, alcohol metabolism is modified with increasing age because of decreasing activity of enzymes used in alcohol metabolism such as ADH, ALDH, and CYP2E1 (Meier and Seitz, 2008).
- **Ethnicity:** Alcohol metabolism can be significantly affected by polymorphism of genes that translate alcohol dehydrogenase and aldehyde dehydrogenase. For instance, East Asians have ALDH2*2 allele and they cannot metabolise acetaldehyde appropriately because of deficient aldehyde dehydrogenase enzyme (Dasgupta, 2015).
- **Alcoholism:** An alcoholic person has a higher alcohol metabolic rate.
- **Drugs:** Elimination rate of alcohol decreases with pyrazoles, isobutyramide which reduce ADH or with methanol, ethylene glycol which keep up with ethanol for ADH (Cederbaum, 2012).

### 2.4.Metabolism

Comprehension of the metabolism of ethanol is important for the prediction and management of the outcomes of its ingestion. Between 90 to 98% of absorbed ethanol is eliminated from the body with the oxidation enzymes. Generally, the excretion of around 2 to 10% ethanol is unaltered, mostly through the lungs and kidneys. Low levels of ethanol can be determined in sweat, tears, bile, gastric juice, saliva, and other secretions. Ethanol is enzymatically oxidized mostly in the liver, and slightly in the kidney. Three enzymatic reactions occur in this metabolic process. In the first step, the oxidation of ethanol to acetaldehyde by the alcohol dehydrogenase enzyme take place. This enzyme needs NAD as a cofactor. This is the rate-determining step in disappearance of alcohol from the body. Catalytic conversion of other primary alcohols and aldehydes are made by hepatic alcohol dehydrogenase.

In the second step, acetaldehyde is transformed to acetic acid. This process takes place with aldehyde dehydrogenase in the presence of NAD. Acetyl coenzyme A (CoA) forms if acetic acid is available. With the entry of Acetyl CoA to the Krebs cycle, it is metabolised to $CO_2$ and $H_2O$.

$$\underset{\text{Ethyl Alcohol}}{C_2H_5OH} + NAD^+ \xrightarrow{\text{Alcohol Dehydrogenase}} \underset{\text{Acetaldehyde}}{CH_3CHO} + NADH_2$$

$$\underset{\text{Acetaldehyde}}{CH_3CHO} + NAD^+ \xrightarrow{\text{Aldehyde Dehydrogenase}} \underset{\text{Acetic Acid}}{CH_3COOH} + NADH_2$$

$$\text{Acetic Acid} \xrightarrow{\text{CoA}} \text{Acetyl CoA} \xrightarrow{\text{via Kreb's cycle}} CO_2 + H_2O$$

**Figure 2** Biochemical pathway for ethanol methabolism (Gossel, 1990)

Ethanol is metabolised with the use of NAD and collection of its reduced form, $NADH_2$. This causes a critical decrease in the NAD/$NADH_2$ ratio in the liver, and some of the metabolic effects of ethanol intoxication depend on this. For instance, hypoglycaemia takes place after acute and chronic ethanol intoxication. It can be tremendously serious in children and is often ignored. Despite the fact that ethanol ingestion has complex metabolic results, it is believed that they are moderately because of inhibition of gluconeogenesis by ethanol because of reduction of NAD. As a result, amino acids that generally goes into glycolysis pathway and tricarboxylic acid cycle derivate into other pathways. Oxaloacetate and pyruvate decreases and lactate and glycerol accumulation reduces, causing collection of fat in the liver (Gossel and Bricker, 1990).

### 2.5.Gastric Emptying (GE)

Gastric emptying is a significant stage in the absorption of foodstuffs. Gastric emptying is a complicated procedure that is managed by different features, for example, neuronal control, hormonal emission, and motility of the stomach. Gastric emptying is affected by several elements, for example, volume, composition, energy ingredient and temperature of the meal, age, sex, physical movement and position of the subject, drug admission, and time (Hauser, 2006).

# 3. MEASUREMENT OF ETHANOL AND GASTRIC EMPTYING

## 3.1.Measurement of Ethanol

Measurement of ethanol can be made in blood, breath or urine with the help of different methods. Gas chromatography, chemical and enzymatic techniques are credible for forensic measurement. Moreover, infra-red spectrophotometry and fuel cell potentiometry are methods for breath measurement.

### 3.1.1. Collection of samples

#### i. Blood

Blood tests are generally acquired by phlebotomy, utilizing a clean, dispensable, empty needle with a sloped straight edge; that is, a hypodermic needle. This is joined to a syringe, likewise dispensable, or an emptied glass test tube shut by an elastic bung. The needle punctures the skin and afterward the barrier of a surface vein, for example, one in the bend of the elbow. The skin is normally cleaned in advance. It is normal practice in medical centre to scrub the skin with a small cushion drenched with an isopropanol, or with cotton wad immersed in an antiseptic, for example, chlorhexidine, which may itself be disintegrated in ethanol. This can cause contamination of the blood test (Dubowski, 1988). Care is thus required for understanding the outcomes about samples taken from patients in medical centre.

Blood tests, once acquired, are kept in or discharged into test-tubes. In the event that investigations are to be made on entire blood or plasma, at that point it is important to stop the blood thickening, and the test-tube will mostly contain potassium oxalate or heparin. Bacterial defilement, which is more hypothetical danger than a demonstrated hazard (Dubowski, 1988), can bring about the production of ethanol from carbohydrate. To prepare for this, sodium fluoride is utilized to hinder enzymic oxidation. It has no impact if tests are put away firmly shut, yet it is useful in the event that they are over and over again opened (Somogyi, cited in Ferner and Norman, 1996). Refrigeration at $4_{o}$C ensures against bacterial development and substance response (Brown, cited in Ferner and Norman, 1996).

The ethanol concentration in entire blood is lower than that in plasma or serum, since red cells include fat, and ethanol is indissoluble in fat and remains in the aqueous liquid. It is hence essential to know which liquid was utilized for investigation (Ferner and Norman, 1996).

#### ii. Urine

Urine specimen are elementarily gathered, but since the bladder can store urine more than a few hours, precision of timing is restricted. The circumstance is enhanced if the subject discharges the bladder and after that in this manner gives the example of new urine. In Britain, a period of time of up to 1 hour after the bladder has been purged is considered the subject to create urine for evidential samples (Walls and Brownlie, 1985).

#### iii. Breath

Breath tests for quantitative investigation are created by blowing into a mouthpiece which is appended to the breath testing machine such that the initial 1.5-2 liters of breath are disposed of, and just the last 50-100 milliliters are permitted into the example chamber. This is critical, on the grounds that the air in the upper piece of the aviation routes does not have the chance to equilibrate with ethanol in the blood, and can't give a dependable measure of blood ethanol concentration (Ferner and Norman, 1996). In spite of the fact that the Road Traffic Act 1962 qualified the courts for the observation of any proof from the examination of blood, urine or breath, the last was never used. It was not until the point when the Road Safety Act 1967 that the utilization of breath testing gadgets was presented and afterward only for road-side screening. Nevertheless, as screening gadgets they provided the police with a capable weapon in the battle against the drink-driver. An officer could capture, without warrant, a man who gave a positive screening test or who declined to take one. The individual captured must be given the chance to take an additional breath test at the police station prior to any prerequisite for a specimen for lab investigation could be made (Emerson, 2004).

### a. Breath Analysis

The key standard of breath test is the dissipation of alcohol from the streaming blood into the air in the lungs when the breathing process occurs.

On its way around the body the blood grabs alcohol from any drink the individual may have ingested as it goes through the barriers of the organs used in digestion process, prominently the stomach and the upper piece of the small bowel. This blood at that point goes through the liver on its way back to the heart, where it is then pumped through the lungs before streaming back to the heart to be delivered around whatever remains of the body [the blood vessel supply].

In the lungs, the interchange of oxygen from the air into the blood, and of the waste material carbon dioxide in the retrograde way, takes place continually when breathing continues. Presently, just as the evaporation of carbon dioxide from the blood into the breath occurs, then so does a little amount of any alcohol that is also present. This procedure is known as gaseous exchange.

The real amount of alcohol that vaporises into the breath relies upon its concentration in the blood. This is known as Henry's Law. For a better comprehension of Henry's Law, after pouring some whisky into a glass and then there is a strong smell of alcohol above it. After adding some water to the whisky, the alcohol concentration decreases; thus, the strength of smell of alcohol above it also decreases. As a result, the determination of actual alcohol concentration in the drink can be figured out by measuring the strength of alcohol in the air. It is precisely this procedure that is happening in the deep part of the lungs, where the breathed air and the blood are close contact. Thus, the level of alcohol in the breath relies upon its amount in the blood.

The connection between the equilibrium blood and breath alcohol concentrations is definite, and the estimation of the actual concentration proportion is near 2:300:1. This as a result implies that breath is around 2,300 times weaker in alcohol concentration than the blood it was in intimate interaction with.

The alcohol vaporization process in the lungs is essentially quick, similarly as in regard of a drink. In order to acquire an accurate alcohol estimation, sampling and analysis of breath must be done with a breath sample which has been in close contact with the blood. This close contact happens most proficiently in the small tubes somewhere down in the lungs, the alveoli, which are isolated from the flowing blood just by a thin, penetrable membrane. In actuality, in this way, the incoming air in the alveoli comes into intimate contact with the flowing blood, and is then breathed out.

One essential thing however, this all implies when carrying out a breath test, deep lung air must be taken when sampling. The level of alcohol in the sample for the analysis will appropriately reflect what is available in the flowing blood, thus causing disability in the brain (Lion Laboratories, n.d.).

### 3.2.MEASUREMENT OF GASTRIC EMPTYING

Absorption of alcohol from the stomach is slow while absorption of alcohol from the small intestine is rapid. Therefore, the absorption rate is measured mostly by stomach emptying rate (Kalant, 1996). Moreover, excretion of alcohol occurs in the expired air and the quantity respired is depend on the arterial blood level as there is always an equilibrium between alveolar air and capillary blood. Therefore, a breathalyser is suitable for the determination of blood alcohol concentration because of the diffusion of alcohol through the alveolar air regarding Henry's Law which states that constant ratio of the ethanol level in the air to the level in the blood at a certain temperature (Finch et al., 1974).

Contrast radiography, respiration methods or radio isotopic techniques are used for the determination of gastric emptying rate. These techniques have disadvantages or limitations. The determination of gross abnormalities can be made by contrast radiography; however, the achievable measurement is only the for the completion of emptying. A direct method is obtained by gastric or duodenal aspiration techniques but these methods are invasive and people usually does not like. Analysis of stomach emptying with scintigraphic methods unavoidably cause exposure to radiation and it is based on the accessibility of gamma camera or rectilinear scanner (Holt et al., 1980).

#### 3.2.1. $^{13}$C-Breath Tests

The final product of digestion of $^{13}$C-labelled xenobiotics is $^{13}CO_2$, which is immediately reabsorbed and breathed out. Estimation of the $^{13}C/^{12}C$ proportion in exhaled breath following the administration of such labelled substrates can thus be utilized to identify the speed and effectiveness of metabolic procedures.

Non-invasive breath tests can be used as important analytical tools in medicine as they can determine specific enzymatic and metabolic capacities in vivo. This has broad uses in the fields of gastroenterology, oncology, herpetology and nourishment control. A $^{13}CO_2$ breath test measures the increase in the levels of $^{13}CO_2$ in exhaled breath after ingestion of a stable $^{13}$C isotope tagged substance and its resulting digestion with a particular capacity or catalyst as a rate determining step. Breath samples are gathered and measured with an instrument, evaluating the phase between ingestion by the patient of the tagged substance and its presence in the exhaled breath (Seahorse Laboratories, 2016). .

#### 3.2.1.1.History of $^{13}C$-breath tests

Improved tracer innovation with the use of stable isotopes in people has empowered the non-invasive examination of metabolic procedures and the evaluation of catalyst activities and, organic functions in vivo. The stable isotope method, fundamentally the utilization of $^{13}C$-marked human substances for diagnostic aims has astoundingly enhanced the gastroenterological analytic range.

Breath tests utilizing the radioactive carbon isotope $^{14}C$ have been created 30 to 40 years ago for analysing the exocrine pancreatic activity, intestinal ingestion and liver activity. With an increase in consciousness of radiation risk $^{14}C$ has been displaced by the stable, non-radioactive carbon isotope $^{13}C$ in the breath analysis. For research in children and pregnant ladies, and for repetitive investigations in adults, the utilization of stable isotopically marked substrates are better and safe. Because of its long half-life which is 5730 years the utilization of $^{14}C$ for diagnostic analysis in people is controversial. New $^{13}C$-breath tests have been produced for future clinical signs such as evaluation of stomach emptying or the orocaecal transit time, bacterial overgrowth in the small bowel or malassimilation (Braden, 2007).

#### 3.2.1.2.Principle for $^{13}C$ Breath Tests

The breath test idea depends on a $^{13}C$-labelled tracer test. This is a particular design of a substrate of a "gate-way" enzyme in a distinct metabolic route. The yield of the substrate can be measured by checking the unidirectional decay to labelled carbon dioxide. Breath samples are gathered in certain time periods. The time determining stage from the admission of the tracer substance to the presence of $^{13}CO_2$ in breath is controlled by the disruption of the substrate in the gastrointestinal lumen, the transport processes, or the enzymatic decay. At last, $^{13}CO_2$ is discharged as the final product of digestion and is breathed out. Breath tests rely upon the theory, that the procedure being referred to determines the release rate of $^{13}CO_2$, in light of the fact that all other metabolic procedures are considerably more rapid or not variable (Braden, 2007).

#### 3.2.1.3.Types of $^{13}C$ Breath Tests

The decision of the $^{13}C$-labelled substrate determines if the $^{13}C$-breath test examines transport, digestion, assimilation, oxidation or enzymatic processes. Today, a huge number of test substrates are accessible for various diagnostic aims (Braden, 2007). For liver function measurements, $^{13}C$ Methacetin substrate is used. $^{13}C$ Sodium Acetate and $^{13}C$ octanoic Acid substrate are used for gastric emptying liquid and solid measurements respectively. Moreover, $^{13}C$ Mixed Triglyceride substrate is utilised for pancreatic function measurement (Seahorse Laboratories, 2016).

#### 3.2.1.4. $^{13}C$-Sodium Acetate Breath Test

$^{13}C$ labelled Sodium Acetate is ingested with a liquid or semi-solid analysis meal in order to determine liquid stomach emptying. After its moving through the stomach, where it cannot be absorbed, the absorption of the test meal occurs in the small intestine and its metabolism occurs in the liver (Goetze et al., 2009). While absorption of some of the labelled carbon takes place in distinct metabolic pathways, entrance of approximately half of them to the body's bicarbonate pool and exhalation of them occurs (Sanaka and Nakada, 2010).

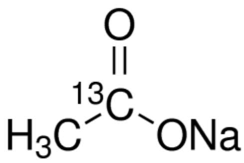


**Figure 3** Structure of $^{13}C$ sodium acetate (Sigma Aldrich,2017)

## 4. MEDICOLEGAL and LEGAL ISSUES WITH ALCOHOL

### 4.1. Effect of Alcohol

How alcohol influences a person is based on:

- the weight, age, sex and metabolism of the person (the rate body consumes energy)
- the kind and quantity of alcohol a person is drinking
- what a person has eaten lately
- the stress levels of the person at the time (GOV.UK, 2017).

Breathalyzer, blood and urine measurements can predict alcohol related behaviours. While individual responses to alcohol differ, the BAC chart below represents the stages and influence of alcohol at different alcohol concentrations.

| BAC mg/dL | Effects |
|---|---|
| < 50 | Little apparent effect, perhaps increased feeling of well-being and talkativeness |

| | |
|---|---|
| 50-100 | Reduced inhibitions, talkativeness, laughter, some loss of co-ordination and sensory perception. |
| 100-150 | More marked loss of co-ordination and sensory perception, slurred speech, lethargy. |
| 150-200 | Obvious drunkenness and possibly nausea |
| 200-300 | Vomiting, general inertia, stupor, inability to stand, possibly coma |
| 300-350 | Anaesthesia, impaired circulation and respiration, danger of aspiration of vomit, possible coma and death. |
| 350-450+ | Progressive danger of death due to respiratory paralysis |

**Table 1** Effect of alcohol at various concentrations

### 4.2.The drink drive limit

There are rigorous alcohol limits for drivers, however; it is not possible to say accurately how many drinks this corresponds to, it can be distinctive for each individual. The limits in Scotland are not same with the rest of the United Kingdom (Table1). There are severe drink driving punishments if a driver is captured over the legal limit. No one cannot drive anywhere in the UK if he or she has been prohibited by any UK court because of driving under the influence of alcohol (GOV.UK, 2017).

| Level of alcohol | England, Wales and Northern Ireland | Scotland |
|---|---|---|
| **Micrograms per 100 millilitres of breath** | 35 | 22 |
| **Milligrammes per 100 millilitres of blood** | 80 | 50 |
| **Milligrammes per 100 millilitres of urine** | 107 | 67 |

**Table 2** Legal limits of level of alcohol for E&W, NI and Scotland (GOV.UK, 2017)

### 4.3.Alcohol related criminal activities

Alcohol-related crime' is a common rather than a lawful expression. Generally, it is used to denote to two principal types of offences:

- Alcohol-defined offences like inebriation offences or driving with excessive amount of alcohol

- Offences in which the utilization of alcohol is thought to have played a part in the perpetrating the offence, generally in a manner that the criminal was affected by alcohol at the time. Cases of offences frequently perpetrated by individuals impaired are assault, rupture of the peace, criminal harm and other public order offences.

It has been assessed that in a group of 100,000 individuals every year, 1,000 individuals will be a subject of alcohol related violent crime. The Coalition Government recorded a diminishment in alcohol fuelled malicious offence among its center needs in its Alcohol Strategy and the present Conservative Government features medications and alcohol as one of the six main drivers of offence in its Modern Crime Prevention Strategy (Institute of Alcohol Studies, 2017).

### 4.4.Medicolegal Problems

Specialists and solicitors are continually encountered with similar problems in prosecution or defend for driving with excess ethanol in the blood or breath. Jones has listed the top 10 plea difficulties utilised by people who are driving under the influence of alcohol in Sweden (Jones, cited in Ferner&Norman, 1996):

i) the hip-flask defence
ii) mixed drinks
iii) breathing of ethanol vapour at work
iv) trauma or disease
v) usage of skin sanitisers including ethanol
vi) asserted mix-up of blood samples
vii) generation of alcohols in the specimen after sampling
viii) interaction between drug and alcohol
ix) ingestion of medicines or tonics including ethanol
x) recovery by combination of fluids that have ethanol.

#### 4.4.1. Hip-flask defence

The hip-flask defence relies upon the subject’s drinking after a charged offense of driving with excessive amount of alcohol in blood, however before he or she experiences a test to decide the concentration of ethanol in breath or blood.

The specialist can be made a request to compute whether the quantity drunk after the episode would represent the outcome, or then again, what the value would have been if the subject had just ingested some expressed, and typically little, quantity before the occurrence.

The amount calculated can demonstrate that there is sensible uncertainty that the litigant's blood ethanol concentration surpassed the legal limit at the time of incident, which is useful for the protection; or that the accused's ethanol amount was still, more likely than not, over the legal limit, notwithstanding when the post-incident utilization is considered. It can likewise demonstrate a disparity between the record given of the overall ethanol utilization and the measured ethanol concentration.

### 4.4.2. Lacing defence

The principle of the lacing defence is the charged's statement that he or she was just drinking dilute ethanol, as wine or beer, for instance, however that a colleague had secretively included more strong ethanol, commonly as spirits, for example, vodka or whisky. In spite of the fact that no longer a barrier in the United Kingdom to the accuse of driving of excessive amount of alcohol in the blood, legal advisors may display proof of lacing to the court to indicate uncommon reasons why their customer ought not be precluded from driving.

It is frequently feasible to demonstrate that the accused would have expected to drink, unprepared, expansive volumes of spirits to deliver the measured ethanol amount (Ferner and Norman, 1996).

One related issue, to be specific whether a respondent could be required to understand that a drink was mixed, has not been formally analysed. A small pilot investigation with different avenues regarding partners proposes that most cannot identify amount of vodka in beer. A related analysis has demonstrated that even determined, experienced, subjects can have the capacity to recognize liquor from whisky (Campbell et al., 1994).

## 5. METHODOLOGY

The aim of this research is to observe the effects, if any, on the pharmacokinetics of alcohol metabolism caused by ingestion with diet coke and with regular coke.

With this aim of the study, the evaluation of breath alcohol concentrations and the rate of gastric emptying using $_{13}$C-Sodium Acetate test were made in healthy volunteers following alcohol consumption with either sucrose containing or artificially sweetened mixers. Based on previous studies, the expected result is that artificially sweetened beverages would empty from the stomach more quickly, causing higher breath ethanol concentrations (Marczinski, 2013).

### 5.1.Study Design

This is an open labelled two-way crossover study. Given the flavour of the soft drink it will not be possible to mask the treatment from the subject so the study will of necessity open label. On two occasions, the participants will consume alcohol with diet coke, or alcohol with regular coke. The order in which the administration occurs will be randomised for each participant. During the three hours following ingestion, breathalyser readings will be taken at regular fixed intervals. Participants will be asked to mark a series of Visual Analogue Scales (VAS) to determine their subjective level of intoxication. From these data, the pharmacokinetics of alcohol will be determined for each session and compared.

### 5.2.Subjects

For this research study, 7 normal volunteers are used. They are over 18 and consumers of drinks containing alcohol and soft drinks at least once per week.

### 5.3.Materials

The amount of alcohol (Smirnoff Red Label Vodka, 37.5% ABV) given will be 0.5g/kg body weight. In a 70kg person, this equates to approximately 3.5 single measures of vodka. The vodka will be mixed with 330mL of diet coke or 330mL of regular coke.

For gastric emptying measurement, 75 mg capsule of Sodium Acetate (1-$_{13}$C) per subject is added to alcohol-coke mixture (Seahorse Laboratories).

### 5.4.Treatment

Prior to each session, the subjects will be required to fast and abstain from soft drinks for at least four hours. During the three hours following ingestion, breathalyser readings will be taken at regular prior to drinking and then every 15 minutes during 3 hours after the alcohol administration. Participants will be asked to mark a series of Visual Analogue Scales (VAS) to determine their subjective level of intoxication every 30 minutes.

## 5.5.Measurements

### 5.5.1. Demographics

The sex, height, weight, and date of birth will be recorded for each subject. Before participating in the study, each participant will be required to fill in a questionnaire on their normal alcohol consumption.

### 5.5.2. Breath alcohol concentrations

Breath alcohol will be measured using a Lion Alcometer SD-400 and 500 hand held breathalyser. It utilises the Lion fuel cell sensor, so the reading is very unlikely to be influenced by anything in the subject's breath other than alcohol. The investigator switches the instrument on, then attaches a new mouthpiece to the sampling port. He or she then instructs the subject to blow through it until the sample is taken. A short time later, their alcohol level is shown on the clear, easy-to-read display (Lion, n.d.).

### 5.5.3. Stomach Emptying

Stomach Emptying will be measured using $^{13}$C-Sodium-Acetate Test. 75 mg of $^{13}$C-Sodium-Acetate is consumed together with liquid meal. After passing through the stomach, where there is no absorption, it is absorbed in the small intestine and processed in the liver. While some of the labelled carbon is integrated in different metabolic pathways, about 50 % enters the body's bicarbonate pool and is exhaled. After collection of breath samples, they are analysed with a IRIS® instrument (Seahorse Laboratories, 2016).

### 5.5.4. VAS

This was evaluated through a visual analogue scale (VAS) including intoxication, alertness, and nausea. This was assessed before and every 30 min after the treatment. Each 10-cm line represented the whole range of possible intensity of each listed feature (e.g. intoxication, alertness, and nausea). The volunteers marked the location that matched to the intensity of their feeling with a vertical line. A sample of VAS is in Appendix 1.

## 5.6.Instrumentation for Breath Analysis

Lion Alcolmeter® SD-400 and Lion Alcolmeter® 500 are used in this study.

### 5.6.1. Lion Alcolmeter® SD-400

The Lion Alcolmeter® SD-400 utilizes an electrochemical fuel cell sensor to quantify the concentration of alcohol vapour in the subject's expired breath; a deliberate measure of which is drawn from the mouthpiece into the power module via the sampling system. In the energy compartment, a voltage is produced proportionally to the breath alcohol concentration: it is in this way opened up and showed regarding the subject's alcohol level. The breath testing system is set in the factory to test deep lung air; it is the breath from the base of the lungs that really represents the quantity of alcohol in the body (Lion, 2012).

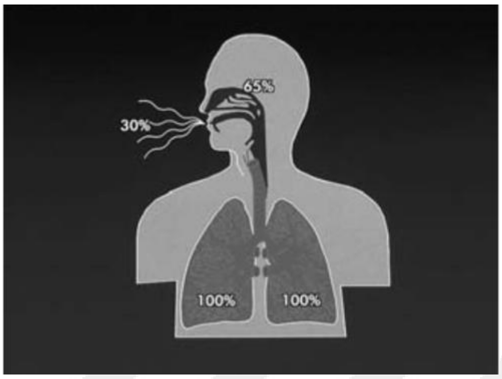



**Figure 4** The breath indicating the amount of alcohol in the body (Lion Laboratories, 2012)

The most important analytical part of all lion alcolmeter® series is the Lion fuel cell sensor. This instrument takes the breath sample, from which any alcohol that is available in it turns out to be immediately absorbed onto its working electrode: this electrode is produced using a specifically arranged platinum-dark catalyst material. On this electrode, a spontaneous oxidation reaction of the alcohol occurs, which so brings about the loss of electrons from its particles. These stream around an outer circuit to the counter electrode, where oxygen from the air experiences a simultaneous decrease, so getting the electrons. This exchange of electrons from the alcohol electrode to the oxygen electrode by means of an outer circuit comprises an electric current. The bigger the quantity of alcohol atoms that were available in the specimen, that is, the more its concentration, then the more electrons that are produced, thus the larger the electric current that streams. Therefore, by measuring the electric current that streams from the alcohol electrode in the power unit, the determination of the concentration of alcohol that must have been available in the sample of breath can be done. The Lion energy unit has a high specificity to alcohols, and, despite the fact that not absolutely ethanol-specific, it is improbable that the breath of a living human could ever contain whatever other synthetic substance to which this sensor reacts (Lion Laboratories, 2017). The process of an analysis for a breath sample is:

1. The breath sample is introduced to the fuel cell.

2. Chemical oxidation of the alcohol in the sample occurs at the anode.
3. Simultaneously, chemical reduction of oxygen (from the air) occurs at the cathode.
4. A current stream, relative to the concentration of alcohol, is generated between the two electrodes.

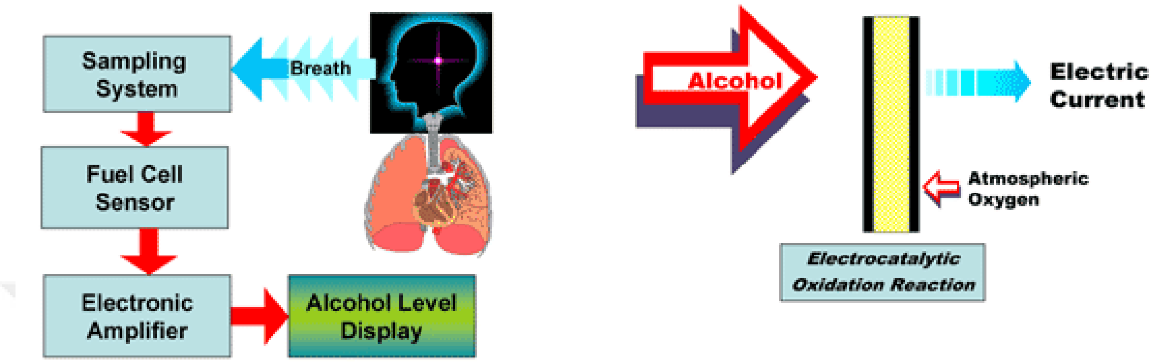



**Figure 5** The working principle of breathalyser (Lion Laboratories, 2017)

### i. User properties of lion alcolmeter® SD-400

**Figure 6** Features of the lion alcolmeter® SD-400 instrument (Lion Laboratories, 2012)

| | |
|---|---|
| **1** | On/Off Switch |
| **2** | Indicator Lights |
| **3** | Digital Screen |
| **4** | Beeper |
| **5** | Sampling port |
| **6** | Spigot Mouthpiece |
| **7** | Function Switches |
| **8** | Communications Port |
| **9** | Power source unit |

**Table 3** Names of the parts of the lion alcolmeter® SD-400 instrument (Lion Laboratories, 2012)

1. **On/Off Switch (I-0):** In order to switch on and off it is necessary to press the button. A person must not use the on/off button due to switching off during a breath test.
2. **Indicator Lights**

- **WAIT:** the system is not set to take a sample; maybe because the instrument is not get rid of alcohol from the previous test.
- **READY:** the device is ready for taking a sample.
- **FLOW:** the subject is blowing sufficiently hard.
- **ANALYSING:** a sample has been obtained: the measurement of alcohol content is being made.

3. **Digital Screen:** It indicates the test subject's alcohol level, time and date information, the battery status, and error messages. It has illumination in order to use when it is dark.
4. **Beeper:** It provides audible messages of definite incidents and circumstances.
5. **Sampling Port:** It is the entrance to the energy unit and breath stream detector. It is necessary to prevent obstruction and blocking.
6. **Spigot Mouthpiece:** A new mouthpiece is attached to the sampling hole for every breath analysis. This single-use material is separately and sanitarily packed.
7. **The Four Function Switches:** They have several usages. From the left, they are denoted to as B, C, and D and the on/off button is A. To check the exactness of the time and date, turn the instrument on and when the READY light is on, push and release B. The time (hh:mm) and at that point the date (dd:mm) are shown. The standard factory-set is that a breath specimen can also be taken physically. To do this, push C while the subject is blowing, but shortly before they come up short on breath - with the goal that deep lung air is taken for investigation. When the READY light is on, push and leave D. The display demonstrates the remaining memory capacity, then the Test Number and the outcome of the previous breath analysis, with its date and time.
8. **Communications Port:** This permits admission to definite software procedures such as calibration correction, when the Dongle key is fixed. If the device is utilised

with a printer, the printer cable is attached here. This port is also used for transmitting collected breath analysis information to a personal computer.

9. **Power Source:** This is situated on the back of the device and attached with either 5 throwaway alkaline batteries (AA) - the PSU; or the rechargeable power unit the RPU. To disconnect from the principal case, press and hold the release switch on the main case, then pull down (Lion Laboratories, 2012).


**Table 4** Subject Breath Test Procedure for lion alcolmeter® SD 400 (Lion Laboratories, n.d)

### 5.6.2. Lion Alcolmeter® 500

This version of the lion alcolmeter is newer than lion alcolmeter® SD-400 in Lion's range of breath alcohol measuring instruments for breath alcohol testing applications in traffic law enforcement, commercial health and safety programs, and in medicine. It is utilised considerably by Police Forces around the world, mostly for detection but also for probative analysis, and is Type Accepted by the Home Office, Great Britain, for UK Police practise in the United Kingdom. This device can also be used with a nasal pipe, for taking breath sample from a patient in a coma for medical purposes (Lion Laboratories, 2015).

**i. User properties of lion alcolmeter®500**

| | |
|---|---|
| **1** | Disposable Mouthpiece |
| **2** | Function Switch A [FSA] Small button |
| **3** | Function Switch B [FSB] Large button |
| **4** | Display |
| **5** | Beeper |
| **6** | Printer, Communications and Charger Port |


**Figure 7** Features of the lion alcolmeter® 500 instrument (Lion Laboratories, 2015)

**Table 5** Names of the parts of lion alcolmeter® 500 instrument (Lion Laboratories, 2015)

1. **Disposable Mouthpiece:** It is provided in an individual, sterile packet. It is connected to the device by confining its sampling port into the small hole in the side of the tube. But the breath sample must be taken from the wide-bore, lipped end.

   **Breath Sampling Port:** The small white hole situated on the upper side of the device is the entry to the fuel cell alcohol sensor and to the breath stream sensor.
2. **Function Switch A:** It is the small button with a red power symbol and situated above the screen. It turns the device on by pressing FSA and releasing. It can be used for switching the device off.
3. **Function Switch B:** It is situated above FSA, which is the bigger button. It is used when the sampling is made manually and also during calibration checking and regulation.
4. **Display:** It is illuminated and it is used for the instruction of the user during data recording and taking a breath sample. It indicates the alcohol reading of the subject.
5. **Beeper:** It creates different audible tone messages to the user, and to the subject.
6. **Printer, Communications and Charger Port:** It is utilised to attach the 500 to the printer. It also provides to download kept breath test data to a computer and for device system design (Lion Laboratories, 2015).


**Figure 8** Simple operating procedure of lion alcolmeter® 500 (Lion Laboratories, n.d.)

### 5.7.Instrumentation for Gastric Emptying Measurement

IRIS®-Doc Autosampler is used in this study.

#### 5.7.1. IRIS®

In this research, IRIS® instrument is used. $^{13}C/^{12}C$ proportion in exhaled breath can be identified by different techniques including mass spectrometry (MS) and non-dispersive infra-red spectroscopy (NDIR). IRIS® is a NDIR instrument that can decide this proportion with a high accuracy 0,2 δ ‰.

##### 5.7.1.1.Principle of IRIS® instrument

IRIS® is a primary instrument for quantitative analysis of breath tests. IRIS® utilizes detectors of non-radioactive $^{13}C$-marked stable isotope depending on infra-red technology. The Infrared Isotope Analyser, IRIS®, evaluates the $^{13}CO_2$ and the $^{12}CO_2$ concentrations from sets of breath samples and relates their proportion to the PDB-$^{13}C$ stable isotope standard. The reproducibility is in ideal conditions superior to 0.2 δ ‰ (IRIS-Doc: 0.4 δ ‰) over an extensive variety of $^{13}C/^{12}C$ stable isotope proportions, and over an extensive variety of $CO_2$ concentrations in breath.

Assessments are made on breath samples as they are taken from the breath sample bags or tubes.


**Figure 9** Breath sample bags (Kibion, 2013)

The IRIS® instrument is accessible in two distinct models, IRIS®-3 and IRIS®-Doc and can be associated with the IRIS®-Multisampler for high throughput testing. The IRIS® Autosampler accepts sixteen (IRIS®-Doc: eight) breath sample bags to be measured in programmed set in two minutes-interval for each sample (Kibion, 2014).

In our study, IRIS®-Doc was used.


**Figure 10** IRIS®-3 and IRIS®-Doc Autosamplers (Kibion, 2013)

### 5.8. Analysis of the measurements

#### 5.8.1. Analysis of breath measurement for gastric emptying

Breath [$^{13}CO_2$] results are generally demonstrated in percentage terms. These terms are the percent dose [$^{13}$C]-recovery per hour, PDR; %dose/h, and the cumulative percent dose [$^{13}$C]-recovery during a determined interval, CPDR; % dose. The PDR values are transformed into the CPDR values with the help of the trapezoidal rule. To obtain more detailed analysis, the fitting of the $^{13}$C recovery percentage per hour curve is made by nonlinear regression to the formula $y = at^{b}e^{-ct}$ with the use of least squares algorithms and the cumulative recovery to the formula $y = m(1-e^{-kt})^{\beta}$. Nonlinear regression analysis of these formulas, where t is time, was accomplished with the commercial software programs (Prism7; GraphPad Software Inc., San Diego, CA) (Excel 16.0 program; Microsoft Corp., Redmond, WA). This analysis gives the constants a, b, c, m, k and β. By using these constants, half-emptying time $T_{1/2b}$ and the lag phase $T_{lag}$ and the gastric emptying coefficient (GEC) are calculated (Braden et al., 1995).

$PDR(t) = at^{b}e^{-ct}$ (Eq.1)

where PDR(t) is the percentage of $^{13}C$ excretion in breath per hour; t is the time in hours; and a, b, and c are constants. The derivation of this formula is the $x^2$- distribution equation, utilised in statistical evaluation. The other formula is given by

$CPDR(t) = m(1-e^{-kt})^{\beta}$ (Eq.2)

where CPDR(t) is the percentage of cumulative $^{13}C$ excretion in breath; t is the time in hours, and m, k, and β are constants with m which is the total cumulative $^{13}C$ recovery when time is infinite. This calculation is originated from the practical fact that the breath test curve indicating the cumulative dose in function of time is contrariwise similar to the scintigraphic curve of stomach emptying.

Nonlinear regression analysis using commercial software programs allows estimation of two distinctive factors of gastric emptying as assessed by $^{13}C$ breath tests. These are the half emptying time and the lag phase:

$t_{1/2}$ defines the time by which half of the total cumulative recovery of the [$^{13}C$] label (CPDR [∞]/2) is achieved and $t_{lag}$ indicates the time at which the [$^{13}CO_2$] excretion rate (PDR[t]) get its maximum value (Sanaka and Nakada, 2010).

$t_{lag} = (\ln(\beta))/k$ (from Eq.2) or b/c (from Eq.1)

$t_{1/2} = (-1/k) \ln(1-2^{-1/\beta})$

### 5.8.2. Analysis of breath measurements

Peak blood ethanol concentrations and the area under the breath ethanol concentration curves are determined.

# 6. RESULTS

## 6.1.Demographics

| Subject number | Age (years) | Height (cm) | Weight (kg) | BMI (kg/$m_2$) | Vodka (mL) | Sex (F/M) | DOB (dd/mm/yy) |
|---|---|---|---|---|---|---|---|
| **01** | 48.3 | 186 | 100.0 | 28.9 | 169.0 | M | 20/04/1969 |
| **02** | 45.3 | 192 | 105.0 | 28.5 | 177.4 | M | 19/04/1972 |
| **03** | 64.2 | 182 | 73.0 | 22.0 | 123.4 | M | 04/06/1953 |
| **04** | 26.8 | 163 | 73.4 | 27.6 | 124.0 | F | 22/11/1990 |
| **05** | 22.1 | 164 | 61.0 | 22.7 | 103.1 | F | 02/07/1995 |
| **06** | 24.9 | 175 | 74.0 | 24.2 | 125.1 | M | 14/10/1992 |
| **07** | 28.7 | 177 | 70.0 | 22.3 | 118.3 | M | 03/12/1988 |
| **MEAN** | 177 | 79.45 | 37.2 | 25.2 | 134.3 | | |
| **SD** | 10.8 | 16.4 | 15.7 | 3.1 | 27.7 | | |
| **MEDIAN** | 177 | 73.4 | 28.7 | 24.2 | 124 | | |
| **MIN** | 163 | 61 | 22.1 | 22 | 103.1 | | |
| **MAX** | 192 | 105 | 64.2 | 28.9 | 177.4 | | |

**Table 6** Demographic information for the subjects & mean, SD, median, min and max values for age, height, weight, BMI and amount of vodka consumed

The average body weight and height for subjects are 74.49 kg (SD = 16.39 kg) and 177 cm (SD = 10.80 cm) respectively. The average BMI for the subjects are 25.17 kg/$m_2$ (SD = 3.06 kg/$m_2$). The average age for subjects is 37.19 years (SD = 15.69 years).

## 6.2.Breath Ethanol Concentrations (µg/L)

| Time (min) | S01 RC | S01 DC | S02 RC | S02 DC | S03 RC | S03 DC | S04 RC | S04 DC | S05 RC | S05 DC | S06 RC | S06 DC | S07 RC | S07 DC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **0** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0 | 0 | 0 | 0 |
| **15** | 360 | 390 | 380 | 440 | 330 | 210 | 260 | 350 | 420 | 350 | 180 | 140 | 280 | 160 |
| **30** | 490 | 500 | 410 | 500 | 330 | 390 | 380 | 400 | 380 | 360 | 360 | 370 | 300 | 360 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **45** | 400 | 460 | 370 | 420 | 330 | 370 | 350 | 420 | 380 | 360 | 320 | 520 | 270 | 360 |
| **60** | 320 | 350 | 320 | 400 | 300 | 340 | 390 | 420 | 380 | 370 | 310 | 540 | 280 | 330 |
| **75** | 260 | 300 | 300 | 340 | 260 | 300 | 380 | 400 | 330 | 340 | 290 | 380 | 270 | 310 |
| **90** | 180 | 270 | 270 | 290 | 210 | 260 | 340 | 410 | 280 | 310 | 280 | 340 | 270 | 250 |
| **105** | 180 | 240 | 270 | 250 | 200 | 240 | 310 | 360 | 240 | 260 | 270 | 280 | 240 | 220 |
| **120** | 170 | 210 | 200 | 240 | 170 | 230 | 280 | 340 | 230 | 240 | 220 | 280 | 200 | 190 |
| **135** | 150 | 190 | 200 | 220 | 160 | 200 | 250 | 310 | 210 | 220 | 220 | 270 | 180 | 180 |
| **150** | 120 | 170 | 190 | 200 | 130 | 200 | 230 | 310 | 200 | 200 | 190 | 240 | 170 | 170 |
| **165** | 100 | 150 | 180 | 160 | 120 | 190 | 220 | 270 | 180 | 170 | 160 | 230 | 160 | 150 |
| **180** | 80 | 140 | 160 | 150 | 110 | 160 | 200 | 260 | 160 | 150 | 160 | 220 | 150 | 160 |

**Table 7** BrAC results for diet coke and regular coke of 7 subjects

The mean peak BrAC was 386 μg/L in the alcohol+regular coke condition compared with 440 μg/L in the alcohol + diet coke condition. Breath alcohol concentrations were significantly greater than after the diet coke than the regular coke. Both the peak breath alcohol concentration and the area under curve were greater after the diet coke (Table 8).

| | $C_{max}$ RC (μg/L) | $C_{max}$ DC (μg/L) | AUC RC | AUC DC |
|---|---|---|---|---|
| **Mean** | 386 | 440 | 448.1 | 507.4 |
| **SD** | 62.9 | 72.3 | 50.8 | 67.6 |

**Table 8** Average $C_{max}$ values with SD for diet and regular coke

No significant difference in $t_{max}$ (time taken to reach peak concentration) and β (rate of elimination) was observed between diet coke and regular coke results.
Statistical evaluation of all results with Minitab software is in the Appendix 2.

**Graphs for breath ethanol measurements**

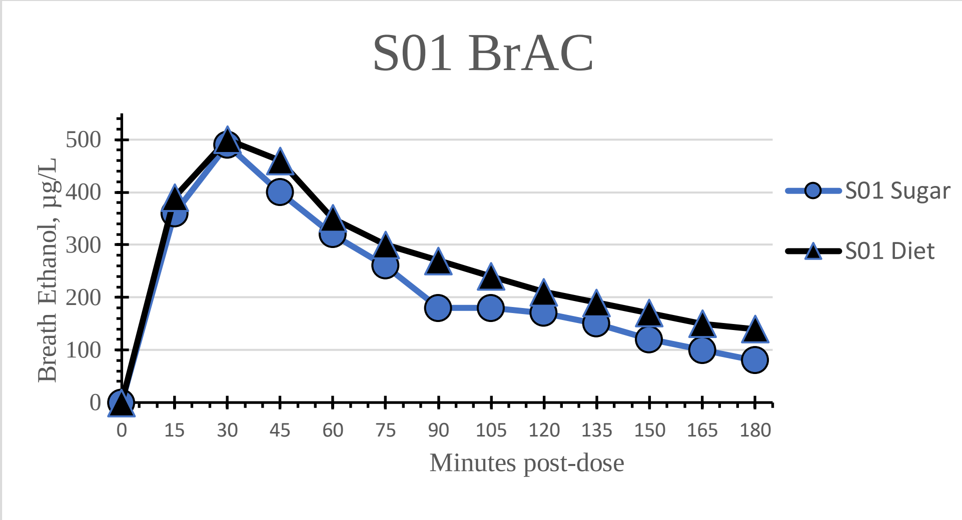



**Figure 11** Breath alcohol concentration curve for Subject 01 for diet and regular coke

$\mathbf{C_{max,\,RC}}$ = 490 µg/L $\mathbf{C_{max,\,DC}}$ = 500 µg/L

$\mathbf{t_{max,\,RC}}$ = 30 min $\mathbf{t_{max,\,DC}}$ = 30 min

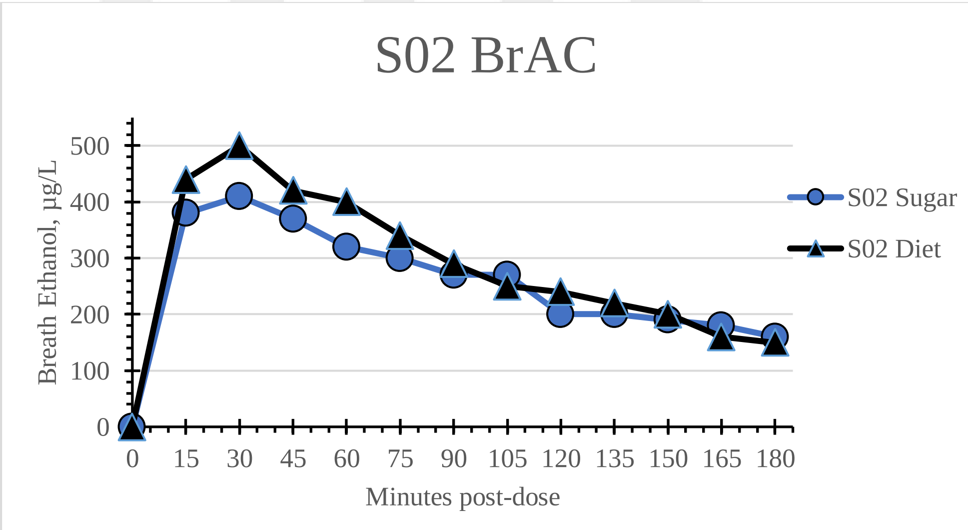



**Figure 12** Breath alcohol concentration curve for Subject 02 for sugar and diet

$\mathbf{C_{max,\,sugar}}$ = 410 µg/L $\mathbf{C_{max,\,diet}}$ = 500 µg/L

$\mathbf{t_{max,\,sugar}}$ = 30 min $\mathbf{t_{max,\,diet}}$ = 30 min

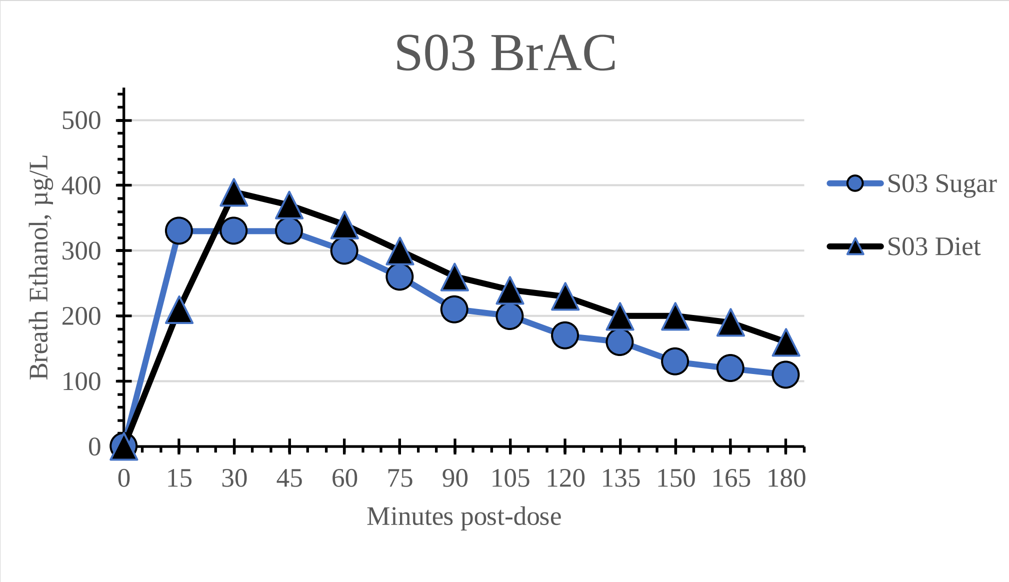



**Figure 13** Breath alcohol concentration curve for Subject 03 for sugar and diet

$\mathbf{C}_{\mathbf{max,\ sugar}}$ = 330 µg/L $\mathbf{C}_{\mathbf{max,\ diet}}$ = 390 µg/L

$\mathbf{t}_{\mathbf{max,\ sugar}}$ = 15 min $\mathbf{t}_{\mathbf{max,\ diet}}$ = 30 min

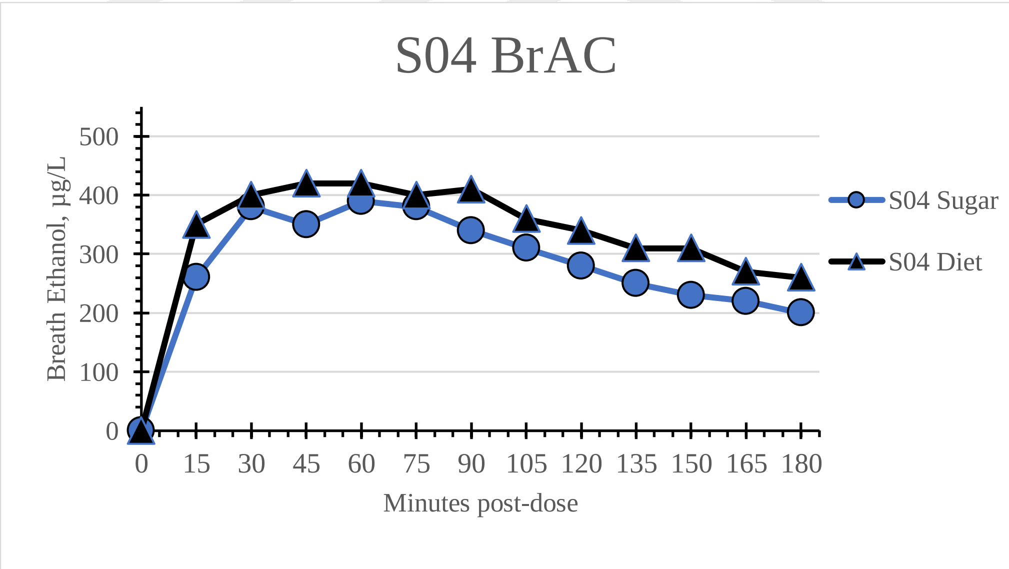



**Figure 14** Breath alcohol concentration curve for Subject 04 for sugar and diet

$\mathbf{C}_{\mathbf{max,\ sugar}}$ = 390 µg/L $\mathbf{C}_{\mathbf{max,\ diet}}$ = 420 µg/L

$\mathbf{t}_{\mathbf{max,\ sugar}}$ = 60 min $\mathbf{t}_{\mathbf{max,\ diet}}$ = 45 min

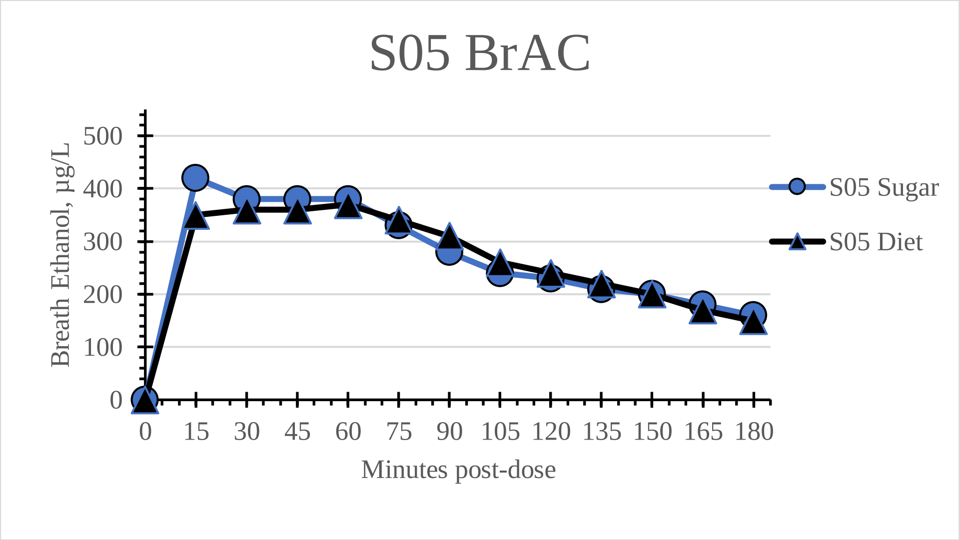



**Figure 15** Breath alcohol concentration curve for Subject 05 for sugar and diet

$C_{max,\ sugar}$ = 420 µg/L $C_{max,\ diet}$ = 370 µg/L

$t_{max,\ sugar}$ = 15 min $t_{max,\ diet}$ = 60 min

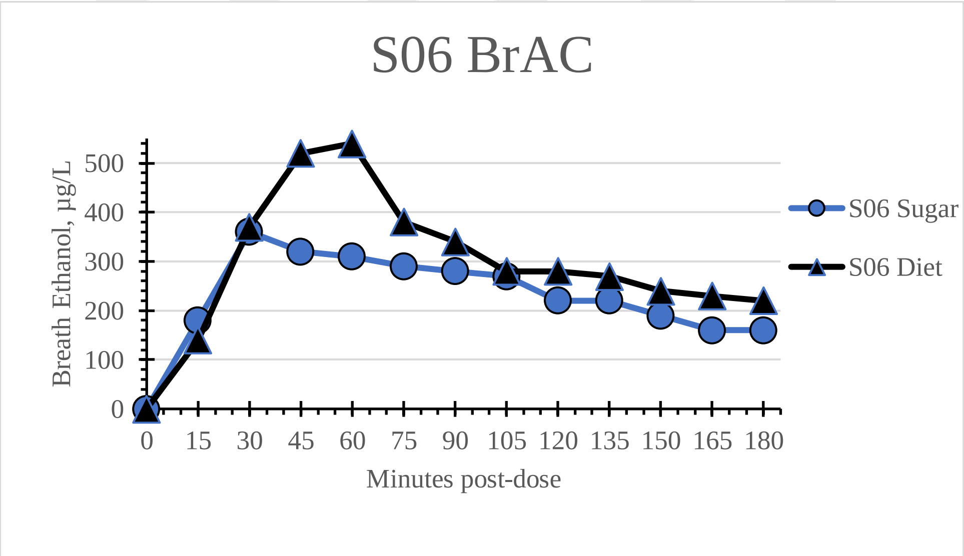



**Figure 16** Breath alcohol concentration curve for Subject 06 for sugar and diet

$C_{max,\ sugar}$ = 360 µg/L $C_{max,\ diet}$ = 540 µg/L

$t_{max,\ sugar}$ = 30 min $t_{max,\ diet}$ = 60 min

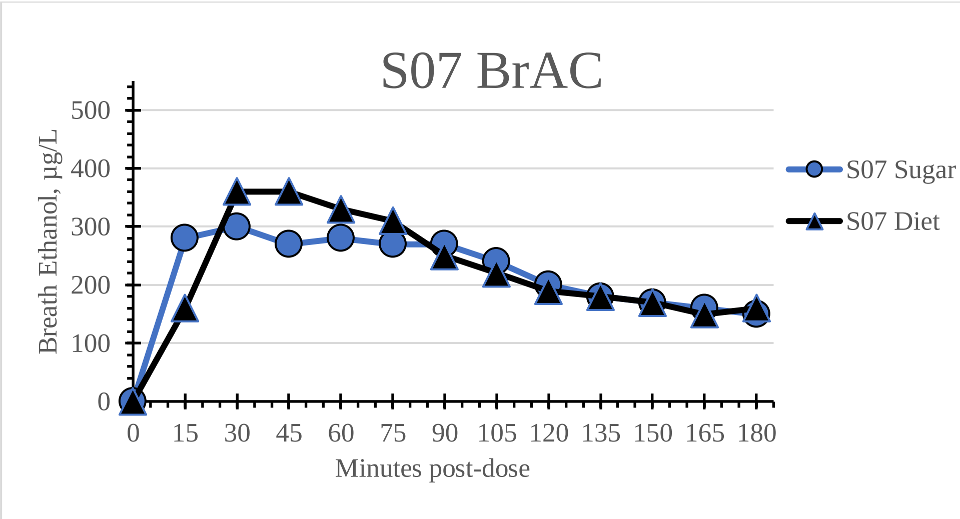



**Figure 17** Breath alcohol concentration curve for Subject 07 for sugar and diet

$\mathbf{C}_{\mathbf{max,\ sugar}}$ = 300 µg/L $\mathbf{C}_{\mathbf{max,\ diet}}$ = 360 µg/L

$\mathbf{t}_{\mathbf{max,\ sugar}}$ = 30 min $\mathbf{t}_{\mathbf{max,\ diet}}$ = 30 min

**VAS Results**

According to VAS results maximum intoxication level is 4.06/10.0 for diet coke which is greater than regular coke, 3.41/10.0. The rate of alcohol absorption has a significant effect on the degree of alcohol intoxication that occurs with acute alcohol intake. Maximum intoxication level was reached at 30 min for all subjects for diet and regular coke. Alertness and nausea values were not changing markedly between diet and regular coke.

Features of the lion alcolmeter® 500 instrument (Lion Laboratories, 2015)

## 6.3.Gastric Emptying Measurements

### 6.3.1. Gastric Emptying (%dose/h)

| Time | S01 RC | S01 DC | S02 RC | S02 DC | S03 RC | S03 DC | S04 RC | S04 DC | S05 RC | S05 DC | S06 RC | S06 DC | S07 RC | S07 DC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **0** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| **15** | 8.8 | 5.8 | 11.8 | 16.2 | 13.1 | 8.4 | 11.4 | 10.5 | 21.8 | 13 | -0.3 | 2.4 | 13.5 | 3.9 |
| **30** | 18.3 | 12.7 | 18.7 | 23.0 | 19.9 | 22.9 | 19.9 | 21.1 | 25.9 | 23.4 | 8.5 | 12.0 | 19.9 | 16.0 |
| **45** | 21.2 | 20.3 | 21.8 | 23.9 | 23.2 | 33.3 | 28.1 | 27.1 | 31.4 | 23.3 | 11.3 | 23.0 | 21.9 | 22.6 |
| **60** | 21.3 | 18.1 | 19.2 | 20.7 | 19.3 | 31.4 | 28.9 | 26.4 | 34.3 | 24.7 | 16.8 | 28.3 | 21.3 | 20.6 |
| **75** | 18 | 16.2 | 17.9 | 20.8 | 18 | 27.8 | 29.8 | 29.7 | 29.7 | 27.2 | 15.9 | 26.0 | 24.0 | 20.4 |
| **90** | 18.3 | 15.2 | 13.8 | 18.8 | 16.7 | 23.6 | 25.4 | 25.1 | 26.1 | 25.3 | 20.0 | 22.2 | 22.6 | 15.6 |
| **105** | 7.5 | 12.4 | 9.2 | 13.3 | 13.9 | 21.7 | 19.9 | 21.9 | 22.5 | 22.5 | 18.6 | 21.2 | 17.2 | 12.7 |
| **120** | 13.2 | 13 | 8.4 | 14.5 | 12.5 | 18.8 | 19.2 | 18.9 | 22.3 | 18.9 | 15.2 | 19.1 | 17.2 | 10.5 |
| **135** | 11.3 | 10.5 | 6.6 | 10.9 | 8.9 | 14.8 | 16.8 | 17.4 | 19.2 | 15.7 | 14.6 | 17.3 | 13.9 | 8.6 |
| **150** | 8.8 | 6.6 | 4.7 | 10.9 | 7.6 | 12.5 | 15.1 | 14.9 | 18.5 | 13 | 12.2 | 13.7 | 10.6 | 9.2 |
| **165** | 9.4 | 6.8 | 3.9 | 11.4 | 5.7 | 10.1 | 14.9 | 13.5 | 14.5 | 11.4 | 10.1 | 11.7 | 12.2 | 5.3 |
| **180** | 6.3 | 4 | 3.4 | 6.3 | 3.3 | 8.1 | 14.5 | 10.7 | 11.5 | 8.9 | 11.1 | 8.9 | 9.4 | 4.2 |

**Table 9** Gastric Emptying results of 7 subjects for Diet Coke and Regular Coke

### 6.3.2. Cumulative Gastric Emptying (cum. %dose/h)

| Time | S01 RC | S01 DC | S02 RC | S02 DC | S03 RC | S03 DC | S04 RC | S04 DC | S05 RC | S05 DC | S06 RC | S06 DC | S07 RC | S07 DC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **0** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| **15** | 8.8 | 5.8 | 11.8 | 16.2 | 13.1 | 8.4 | 11.4 | 10.5 | 21.8 | 13.0 | -0.3 | 2.4 | 13.5 | 3.9 |
| **30** | 27.1 | 18.5 | 30.5 | 39.2 | 33.0 | 31.3 | 31.3 | 31.6 | 47.7 | 36.4 | 8.2 | 14.4 | 33.4 | 19.9 |
| **45** | 48.3 | 38.8 | 52.3 | 63.1 | 56.2 | 64.6 | 59.4 | 58.7 | 79.1 | 59.7 | 19.5 | 37.4 | 55.3 | 42.5 |
| **60** | 69.6 | 56.9 | 71.5 | 83.8 | 75.5 | 96.0 | 88.3 | 85.1 | 113.4 | 84.4 | 36.3 | 65.7 | 76.6 | 63.1 |
| **75** | 87.6 | 73.1 | 89.4 | 104.6 | 93.5 | 123.8 | 118.1 | 114.8 | 143.1 | 111.6 | 52.2 | 91.7 | 100.6 | 83.5 |
| **90** | 105.9 | 88.3 | 103.2 | 123.4 | 110.2 | 147.4 | 143.5 | 139.9 | 169.2 | 136.9 | 72.2 | 113.9 | 123.2 | 99.1 |
| **105** | 113.4 | 100.7 | 112.4 | 136.7 | 124.1 | 169.1 | 163.4 | 161.8 | 191.7 | 159.4 | 90.8 | 135.1 | 140.4 | 111.8 |
| **120** | 126.6 | 113.7 | 120.8 | 151.2 | 136.6 | 187.9 | 182.6 | 180.7 | 214.0 | 178.3 | 106.0 | 154.2 | 157.6 | 122.3 |
| **135** | 137.9 | 124.2 | 127.4 | 162.1 | 145.5 | 202.7 | 199.4 | 198.1 | 233.2 | 194.0 | 120.6 | 171.5 | 171.5 | 130.9 |
| **150** | 146.7 | 130.8 | 132.1 | 173.0 | 153.1 | 215.2 | 214.5 | 213.0 | 251.7 | 207.0 | 132.8 | 185.2 | 182.1 | 140.1 |
| **165** | 156.1 | 137.6 | 136.0 | 184.4 | 158.8 | 225.3 | 229.4 | 226.5 | 266.2 | 218.4 | 142.9 | 196.9 | 194.3 | 145.4 |
| **180** | 162.4 | 141.6 | 139.4 | 190.7 | 162.1 | 233.4 | 243.9 | 237.2 | 277.7 | 227.3 | 154.0 | 205.8 | 203.7 | 149.6 |

**Table 10** Cumulative Gastric Emptying results of 7 subjects for DC and RC

## Graphs for gastric emptying measurements

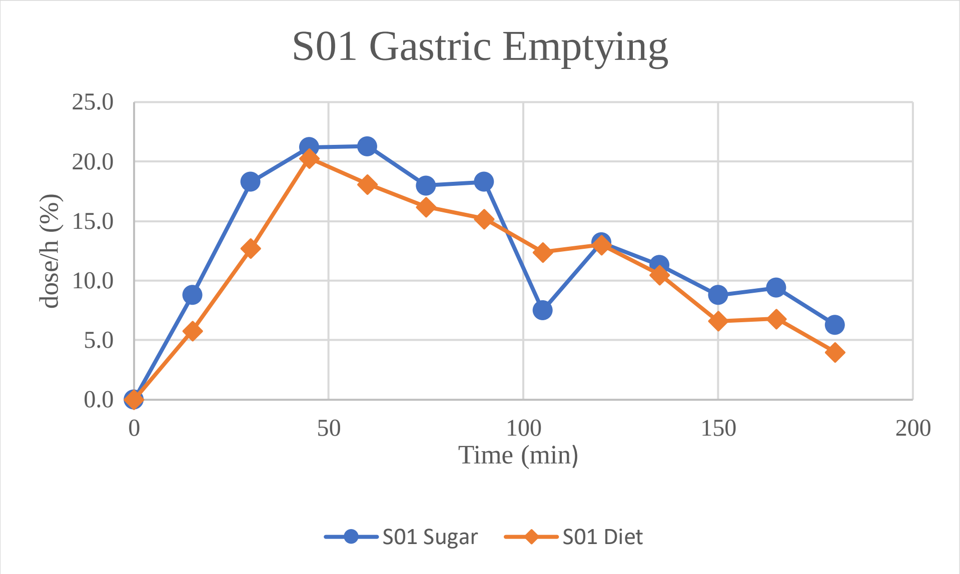



**Figure 18** Gastric emptying curve for Subject 01

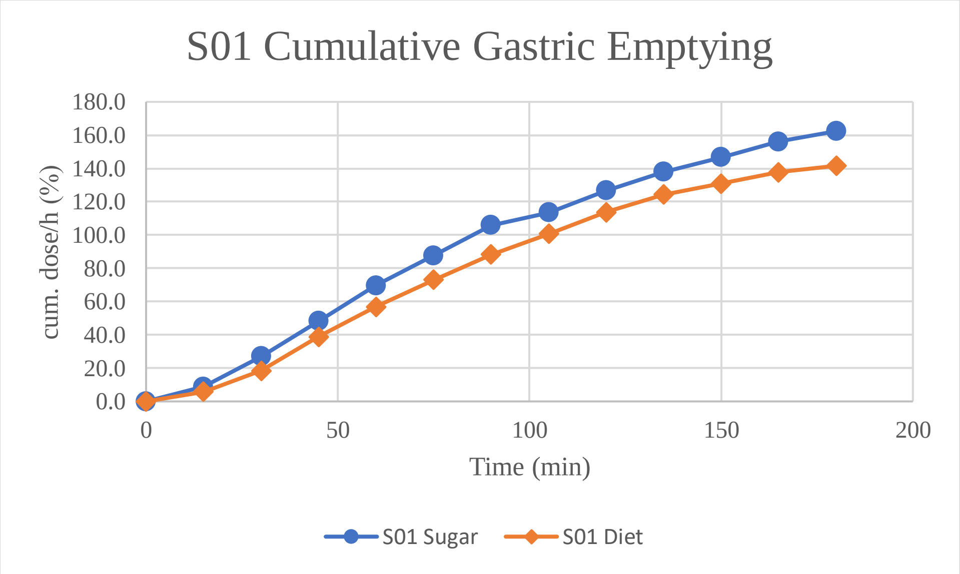



**Figure 19** Cumulative gastric emptying curve for Subject 01

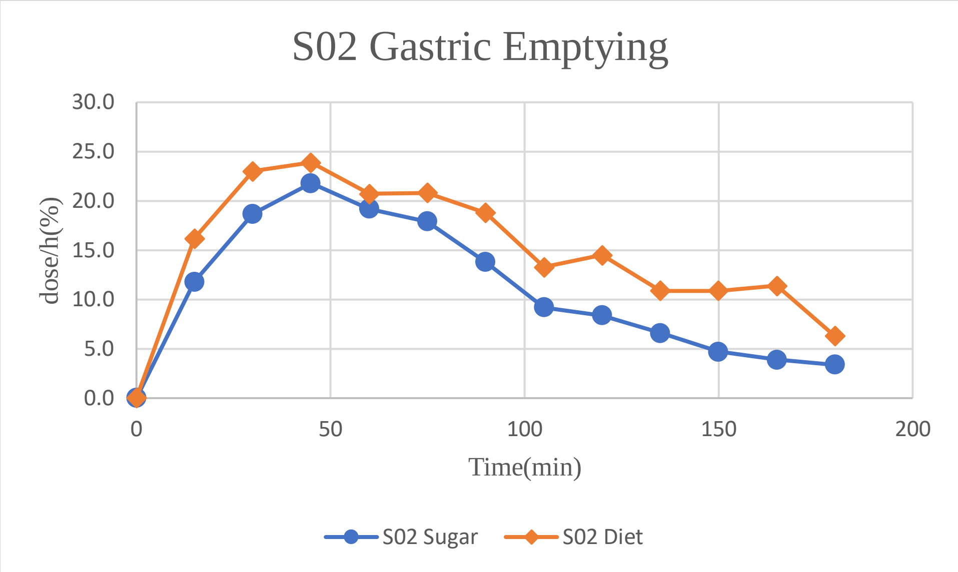



**Figure 20** Gastric emptying curve for Subject 02

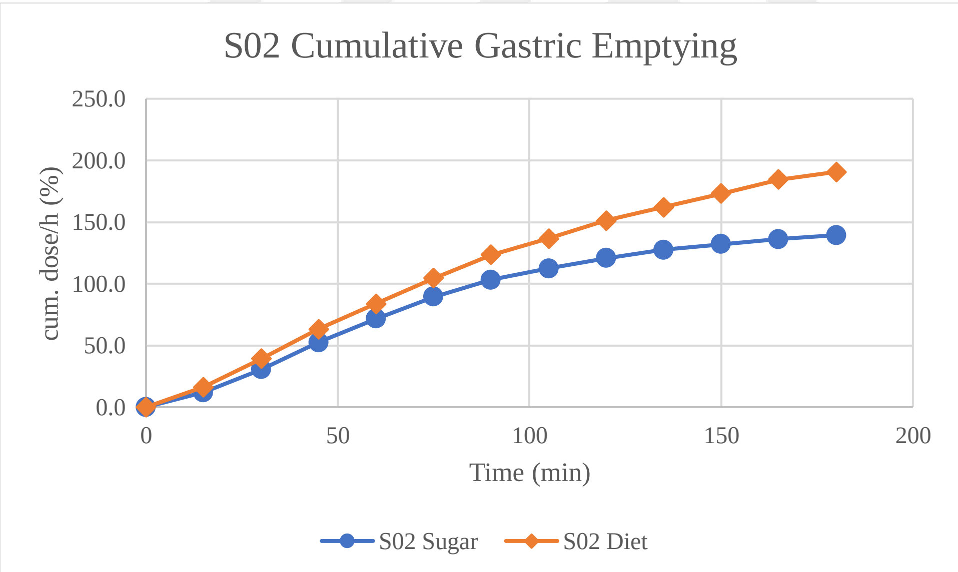



**Figure 21** Cumulative gastric emptying curve for Subject 02

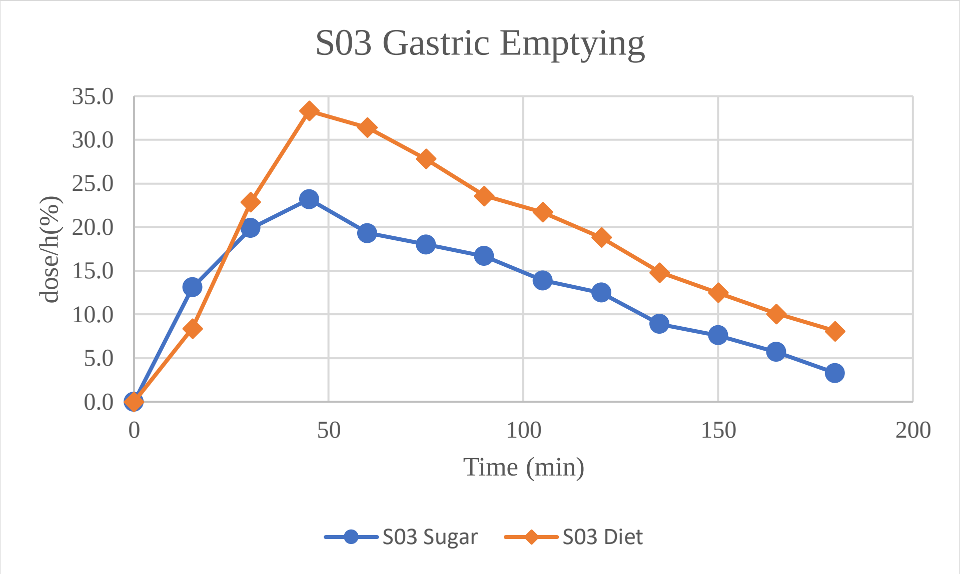



**Figure 22** Gastric emptying curve for Subject 03

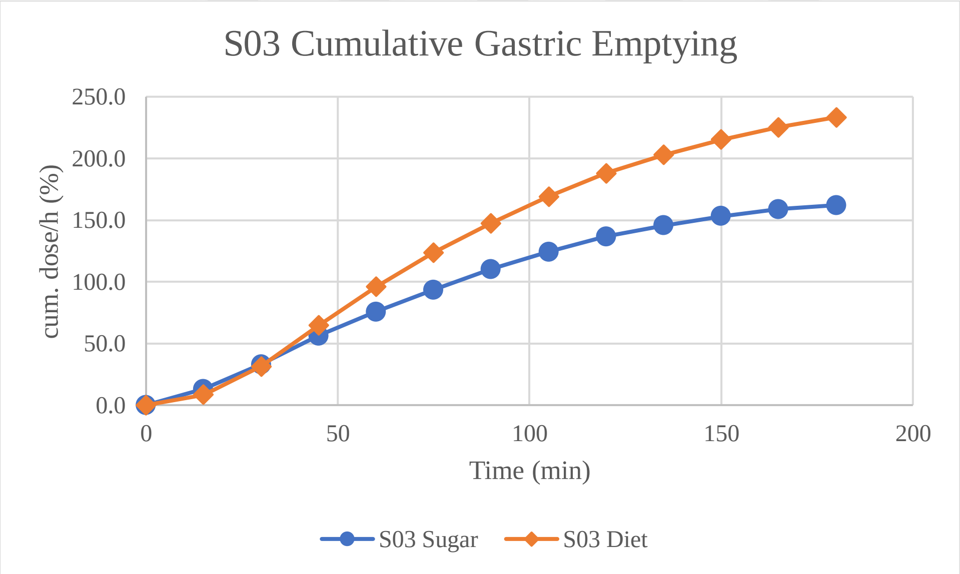



**Figure 23** Cumulative gastric emptying curve for Subject 03

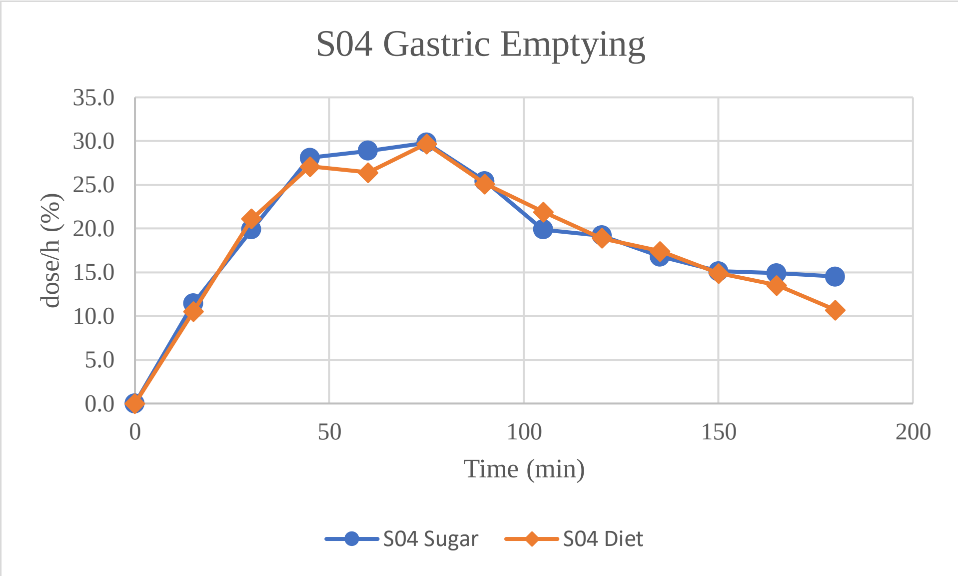



**Figure 24** Gastric emptying curve for Subject 04

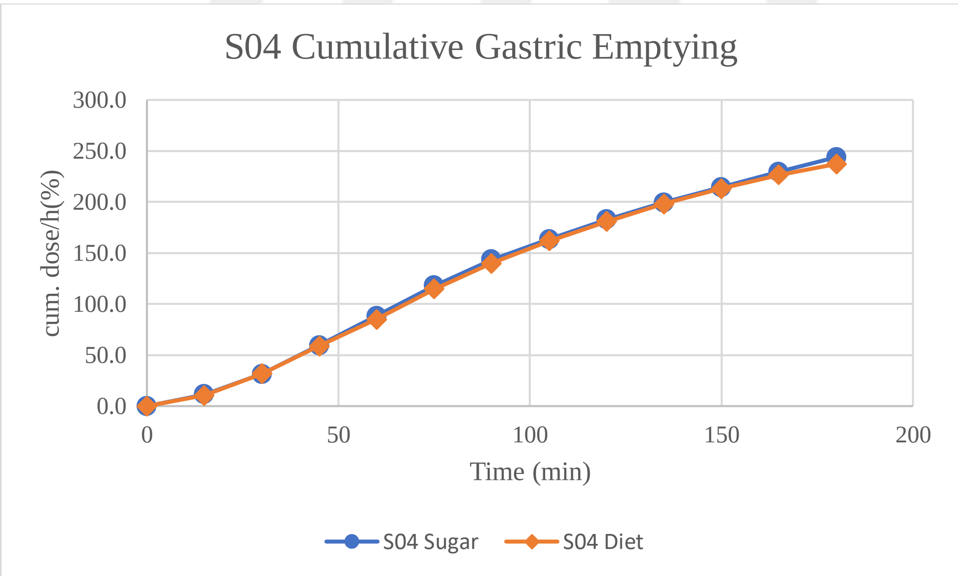



**Figure 25** Cumulative gastric emptying curve for Subject 04

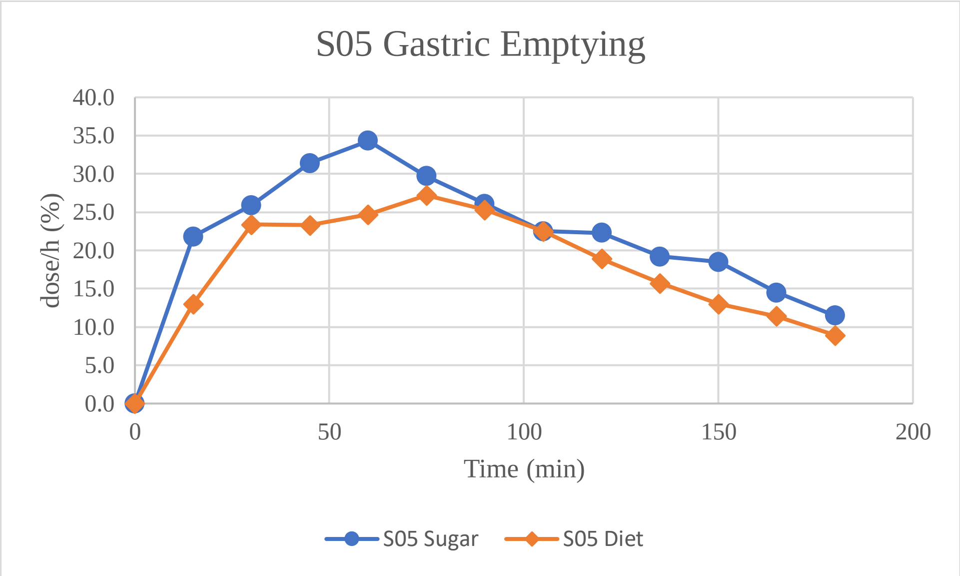



**Figure 26** Gastric emptying curve for Subject 05

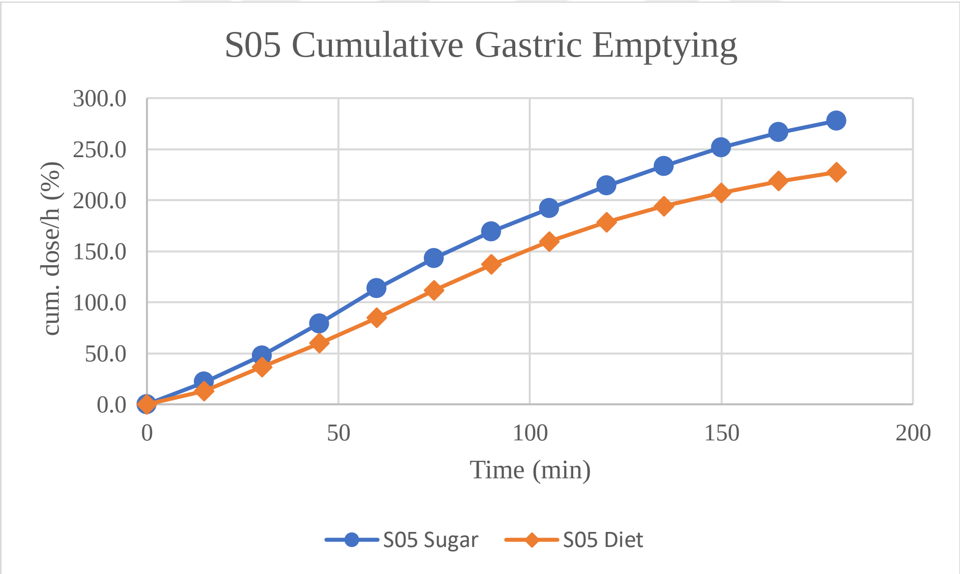



**Figure 27** Cumulative gastric emptying curve for Subject 05

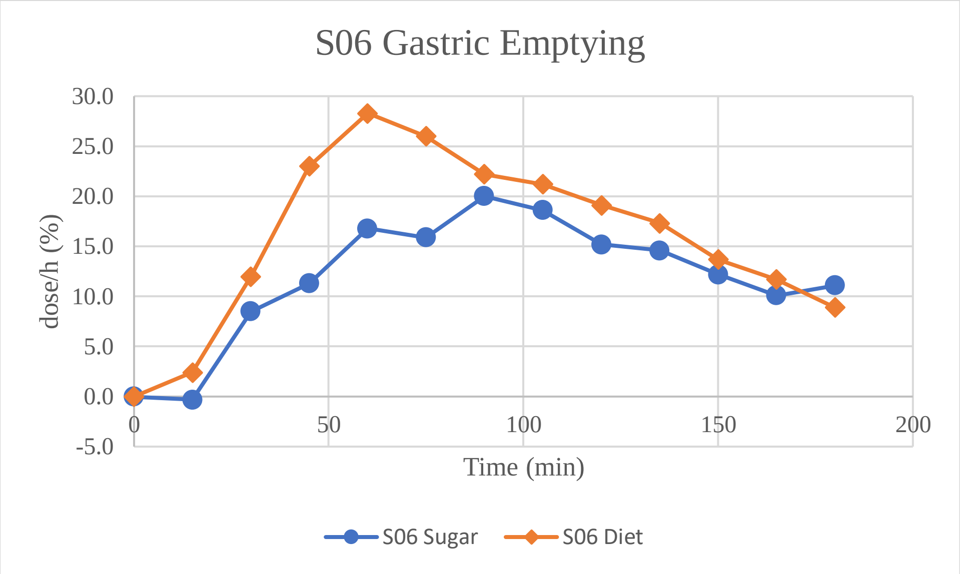



**Figure 28** Gastric emptying curve for Subject 06

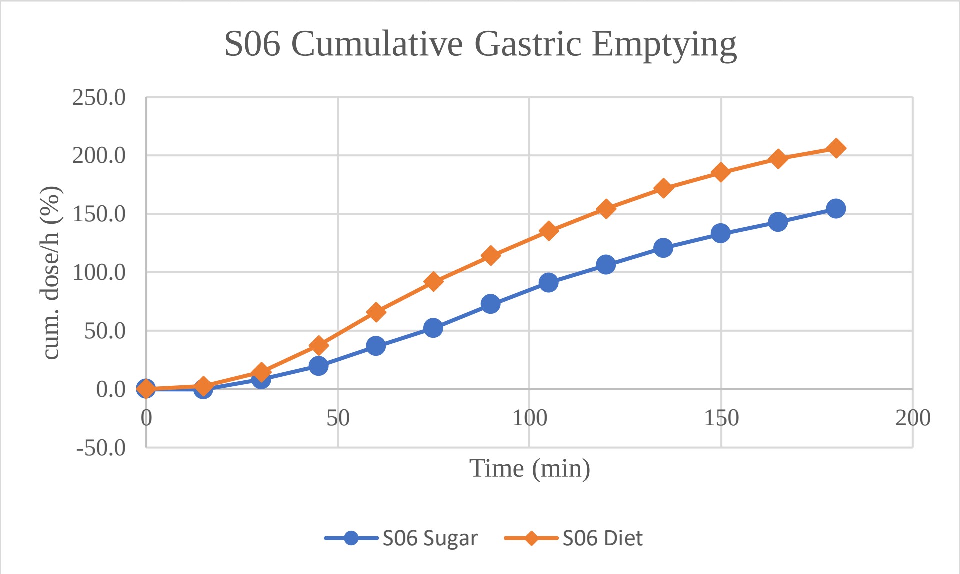



**Figure 29** Cumulative gastric emptying curve for Subject 06

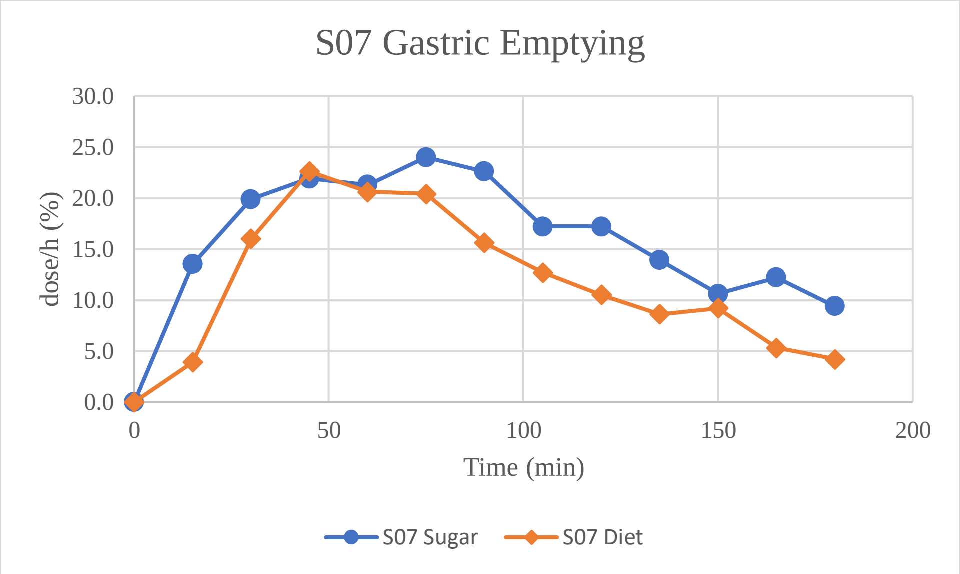



**Figure 30** Gastric emptying curve for Subject 07

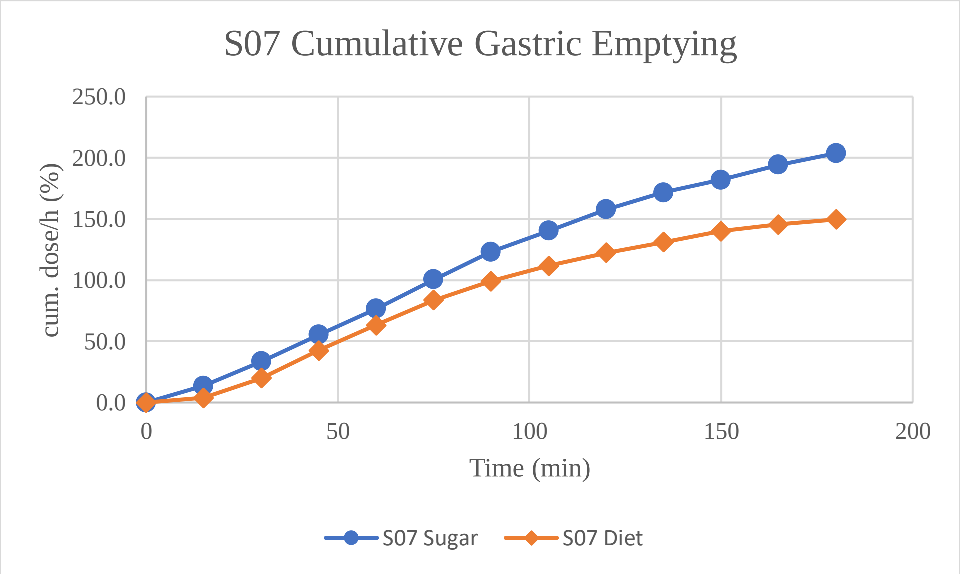



**Figure 31**Cumulative gastric emptying curve for Subject 07

From cumulative gastric emptying values, m, k, and β values are derived with nonlinear regression analysis in GraphPad Software.

| | **m** | **k** | **β** |
|---|---|---|---|
| **S01 Sugar** | 190.1 | 0.01321 | 1.702 |
| **S01 Diet** | 163 | 0.01542 | 2.117 |
| **S02 Sugar** | 147.6 | 0.01927 | 1.894 |
| **S02 Diet** | 236.8 | 0.01075 | 1.389 |
| **S03 Sugar** | 184.4 | 0.01486 | 1.678 |
| **S03 Diet** | 262.2 | 0.01627 | 2.165 |
| **S04 Sugar** | 300.8 | 0.01213 | 1.855 |
| **S04 Diet** | 291.4 | 0.01269 | 1.927 |
| **S05 Sugar** | 360.2 | 0.01025 | 1.499 |
| **S05 Diet** | 280.8 | 0.01242 | 1.815 |
| **S06 Sugar** | 195.8 | 0.01426 | 3.081 |
| **S06 Diet** | 240.6 | 0.01604 | 2.767 |
| **S07 Sugar** | 258.6 | 0.01115 | 1.65 |
| **S07 Diet** | 162.1 | 0.01868 | 2.407 |

**Table 11** m, k, and β constant values derived from nonlinear regression analysis

$t_{1/2}$ and $t_{lag}$ is calculated with these equations using m, k, and β constants:

$t_{lag}$ = (ln (β))/k (from Eq.2) or b/c (from Eq.1)

$t_{1/2}$ = (-1/k) ln (1-$2^{-1/β}$)

| | **$t_{1/2}$ (min)** | **$t_{lag}$ (min)** |
|---|---|---|
| **S01 Sugar** | 82.9 | 40.3 |
| **S01 Diet** | 82.7 | 48.6 |
| **S02 Sugar** | 61.4 | 33.1 |
| **S02 Diet** | 86.9 | 30.6 |
| **S03 Sugar** | 72.9 | 34.8 |
| **S03 Diet** | 79.6 | 47.5 |
| **S04 Sugar** | 96.1 | 50.9 |
| **S04 Diet** | 94.3 | 51.7 |
| **S05 Sugar** | 96.9 | 39.5 |
| **S05 Diet** | 92.4 | 48.0 |
| **S06 Sugar** | 112.4 | 78.9 |
| **S06 Diet** | 93.9 | 63.5 |
| **S07 Sugar** | 96.0 | 44.9 |
| **S07 Diet** | 74.2 | 47.0 |

**Table 12** $t_{1/2}$ and $t_{lag}$ values for each subjects for diet and regular coke

| | $t_{1/2}$ Sugar | $t_{1/2}$ Diet | $t_{lag}$ Sugar | $t_{lag}$ Diet |
|---|---|---|---|---|
| **Mean** | 88.4 | 86.3 | 46.1 | 48.1 |
| **SD** | 17.1 | 7.8 | 15.7 | 9.6 |

**Table 13** Mean and standard deviation values for $t_{1/2}$ and $t_{lag}$

## 7. DISCUSSION

This study establishes that replacement of a diet mixer for a regular one including sugar, in a mixed alcoholic drink, has a significant effect on the rate of stomach emptying and alcohol absorption in healthy people. Despite the fact that we expected these effects from previous researches showing that alcohol absorption depends on gastric emptying, the amount of the difference in breath alcohol concentrations was remarkable. Actually, the mean peak ethanol concentration after the diet drink would prevent subjects from lawfully driving a motor vehicle in several areas, while this action may be lawful after drinking regular beverage.

For regular Coke, sugar slows gastric emptying and therefore the absorption of alcohol into the blood circulation, therefore the alcohol concentration in the blood rises slowly. Diet coke, on the other hand, contains no sugar, only aspartame, which is not handled as food by the digestive system. This leaves the alcohol in the drink to get absorbed more rapidly. Since the subjective feeling of intoxication is related to the rate of rise in blood alcohol concentration[1] this can make people less “drunk” if alcohol is mixed with regular Coke than if it is mixed with Diet Coke or Coke Zero. Therefore, blood and breath alcohol concentrations of a person consumed diet mixers are greater than for a person consumed regular mixers (Wu et. al, 2006).

Our results are coherent with those of Wu et al, who reported that both the peak ethanol concentration and the area under the ethanol concentration curve were greater after the diet drink. Wu et. al also reported that the gastric half-emptying time was less than for the regular drink.

That the rate of stomach emptying is an important factor for alcohol absorption is possibly because of larger surface area for absorption provided by the small bowel when a

[1] It is explained by the Mellanby effect. The Mellanby effect is named for E. Mellanby in 1919 and he stated that the behavioural impairment at a given blood alcohol concentration was greater when the blood alcohol concentration was increasing than when it was decreasing (Mellanby, 1919)

comparison is made with the stomach; alcohol metabolism in the stomach with the help of gastric alcohol dehydrogenase (ADH) is nearly definitely insignificant. Furthermore, we observed that the area under the concentration curve was significantly greater, consistent with the theory of fast absorption because of accelerated stomach emptying (Kechagias et al, 1999). Changes in the gastric emptying rate, which may have a physiologic, pharmacologic or pathologic cause, significantly affect the rate of alcohol absorption. Furthermore, stress, drinking environment, mood, sleep and illness can affect the alcohol absorption and cause differences in alcohol concentration.

## 8. CONCLUSION

This study emphasizes that elements other than just the alcohol content of a drink should be taken into consideration in bearing in mind safe quantities of ingestion and the possibility for intoxication. Particularly, the replacement of artificial sweeteners for sugars in mixed alcoholic beverages possibly has a great influence on alcohol concentrations, even though the beverages being analogous in all other matters. The lack of sucrose in diet mixers might results in quicker gastric emptying of alcohol, causing an increase in its absorption rate into the blood, producing larger peak inebriation and greater exposure to various alcohol related hazards.

## 9. REFERENCES

BRADEN, B., ADAMS, S., DUAN, L. P., ORTH, K. H., MAUL, F. D., LEMBCKE, B., HÖR, G. & CASPARY, W. F. 1995. The [13C]acetate breath test accurately reflects gastric emptying of liquids in both liquid and semisolid test meals. *Gastroenterology,* 108**,** 1048-55.

BRADEN, B., LEMBCKE, B., KUKER, W. & CASPARY, W. F. 2007. 13C-breath tests: current state of the art and future directions. *Dig Liver Dis,* 39**,** 795-805.

BURNING TREE REHABILITATION.2013. *Diet Coke and Alcohol: BAC Overload?* [Online]. Available: https://www.burningtree.com/diet-coke-alcohol-bac-overload/ [Accessed 11 June 2017].

CAMPBELL, E. J., CAMPBELL, D. M. & ROBERTS, R. S. 1994. Ability to distinguish whisky (uisge beatha) from brandy (cognac). *BMJ,* 309**,** 1686-8.

CEDERBAUM, A. I. 2012. Alcohol metabolism. *Clin Liver Dis,* 16**,** 667-85.

DASGUPTA, A. 2015. *Alcohol and Its Biomarkers: Clinical Aspects and Laboratory Determination,* USA, Elsevier, 37-63.

DUFOUR, M., ARCHER, L. & GORDIS, E. 1992. Alcohol and the elderly. *Clinics in Geriatric Medicine,* 8**,** 127-141.

EMELDA, M. 2011. *Difference between Regular and Diet Coke* [Online]. Available: http://www.differencebetween.net/object/comparisons-of-food-items/difference-between-regular-and-diet-coke/ [Accessed 28 August 2017].

EMERSON, V. 2004. Alcohol Analysis. *In:* WHITE, P. (ed.) *Crime Scene to Court The Essentials of Forensic Science.* 2nd ed. Cambridge: The Royal Society of Chemistry, 350-376.

FERNER, R. E. & NORMAN, E. 1996. *Forensic pharmacology : medicines, mayhem and malpractice,* Oxford, Oxford University Press, 113-133.

FINCH, J. E., KENDALL, M. J. & MITCHARD, M. 1974. An assessment of gastric emptying by breathalyser. *Br J Clin Pharmacol,* 1**,** 233-6.

GOETZE, O., FOX, M., KWIATEK, M. A., TREIER, R., SCHWIZER, W., THUMSHIRN, M., FRIED, M. & FRUEHAUF, H. 2009. Effects of postgastric 13C-acetate processing on measurement of gastric emptying: a systematic investigation in health. *Neurogastroenterol Motil,* 21**,** 1047-e85.

GOSSEL, T. A. & BRICKER, J. D. 1990. *Principles of clinical toxicology,* New York, Raven Press, 65-70.

GOV.UK. 2017. *The drink drive limit* [Online]. Available: https://www.gov.uk/drink-drive-limit [Accessed 27 August 2017].

HAUSER, B., DE SCHEPPER, J., CAVELIERS, V., SALVATORE, S., SALVATONI, A. & VANDENPLAS, Y. 2006. Variability of the 13C-acetate breath test for gastric emptying of liquids in healthy children. *J Pediatr Gastroenterol Nutr,* 42**,** 392-397.

HOLT, S., STEWART, M. J., ADAM, R. D. & HEADING, R. C. 1980. Alcohol absorption, gastric emptying and a breathalyser. *Br J Clin Pharmacol,* 9**,** 205-208.

INSTITUTE OF ALCOHOL STUDIES. 2017. *Alcohol related crime in the UK: what do we know?* [Online]. Available: http://www.ias.org.uk/Alcohol-knowledge-centre/Crime-and-social-impacts/Factsheets/Alcohol-related-crime-in-the-UK-what-do-we-know.aspx#_edn2 [Accessed 25 August 2017].

KALANT, H. 1996. Pharmacokinetics of Ethanol: Absorption, Distribution, and Elimination. *In:* BEGLEITER, H. & KISSIN, B. (eds.) *The Pharmacology of Alcohol and Alcohol Dependence.* New York ; Oxford: Oxford University Press, 15-52.

KECHAGIAS, S., JÖNNSON, K.-A. & JONES, A. W. 1999. Impact of gastric emptying on the pharmacokinetics of ethanol as influenced by cisapride. *British Journal of Clinical Pharmacology,* 48**,** 728-732.

KIBION 2013. IRIS®- the reliable instrument for breath test analysis. [Online]. Available: https://www.biovendor.cz/download/6195/Letak_4_WT%208011.pdf [Accessed 28 August 2017].

KIBION 2014. IRIS® and 13C-Breath Tests for the Assessment of Specific Enzymatic and Metabolic Functions in vivo. [Online]. Available: http://www.tareqco.com/images/pdf/22proApplication-brochure.pdf [Accessed 2 September 2017].

LION LABORATORIES. 2015. lion alcolmeter® 500 [500 Instrument (Mk IV) with Printer Option] User Handbook. [Online]. Available: http://www.lionlaboratories.com/preview/wp-content/uploads/SD-400-Ver-7-12-06-13-RM40977.pdf [Accessed 1 August 2017].

LION LABORATORIES. 2017. *Testing for Alcohol* [Online]. Available: http://www.lionlaboratories.com/testing-for-alcohol/?doing_wp_cron=1504284431.0599360466003417968750 [Accessed 30 August 2017].

LION LABORATORIES. 2012. *Lion alcolmeter SD-400 User Handbook: General* [Online]. Available: http://www.lionlaboratories.com/preview/wp-content/uploads/SD-400-Ver-7-12-06-13-RM40977.pdf [Accessed 1 August 2017].

LION LABORATORIES. n.d. Lion Alcolmeter® SD-400 [Brochure] [Online] Available: http://www.lionlaboratories.com/preview/wp-content/uploads/SD-400.pdf [Accessed 20 August 2017].

MARCZINSKI, C. A. & STAMATES, A. L. 2013. Artificial sweeteners versus regular mixers increase breath alcohol concentrations in male and female social drinkers. *Alcohol Clin Exp Res,* 37**,** 696-702.

MCGOVERN, C., PAYNE-JAMES, J., MCGOVER, C., JONES, R., KARCH, S. B. & MANLOVE, J. 2011. *Simpson's forensic medicine*, 217-219.

MCGRATH, B. 2013. Common alcohol mix more risky. *Iowa State Daily*. [Online] Available: http://lib.dr.iastate.edu/cgi/viewcontent.cgi?article=1007&context=iowastatedaily_2013-03 [Accessed 15 August 2017].

MEIER, P. & SEITZ, H. K. 2008. Age, alcohol metabolism and liver disease. *Curr Opin Clin Nutr Metab Care,* 11**,** 21-26.

MELLANBY, E. 1919. Alcohol: Its Absorption into and Dissappearance from the Blood under Different Conditions. *In:* COMMITTEE, M. R. (ed.). London, 1-48.

NHS DIGITAL. 2017. Statistics on Alcohol England,2017. [Online] Available: http://digital.nhs.uk/pubs/alcohol17 [Accessed 20 August 2017].

SANAKA, M. & NAKADA, K. 2010. Stable isotope breath tests for assessing gastric emptying: A comprehensive review. *J Smooth Muscle Res,* 46**,** 267-280.

SEAHORSE LABORATORIES. 2016. *13C-Breath Tests for the Assessment of Specific Enzymatic and Metabolic Functions in vivo* [Online]. Available: https://goo.gl/7RzbTA [Accessed 2 August 2017].

The structure of 13C sodium acetate. (2017). [Image] Available at: http://www.sigmaaldrich.com/catalog/product/aldrich/279293?lang=en&region=GB [Accessed 1 Sep. 2017].

THE COCA-COLA COMPANY. 2017. *Brands* [Online]. Available: http://www.coca-cola.co.uk/drinks [Accessed 1 September 2017].

WALLS, H. J. & BROWNLIE, A. R. 1985. *Drink, drugs and driving,* London, Sweet & Maxwell.

WU, K. L., CHAIKOMIN, R., DORAN, S., JONES, K. L., HOROWITZ, M. & RAYNER, C. K. 2006. Artificially sweetened versus regular mixers increase gastric emptying and alcohol absorption. *Am J Med,* 119**,** 802-804.

**APPENDIX 1**
VISUAL ANALOGUE SCALES

## Intoxication

| Completely sober | | As drunk as I can imagine being |
|---|---|---|

## Alertness

| I am wide awake | | I cannot keep awake |
|---|---|---|

## Nausea

| Not at all nauseous | | Extremely nauseous |
|---|---|---|

# APPENDIX 2

**General Linear Model: Cmax versus Subject, Treatment**

**Method**

| | |
|---|---|
| Factor coding | (-1, 0, +1) |

**Factor Information**

| Factor | Type | Levels | Values |
|---|---|---|---|
| Subject | Fixed | 7 | 1, 2, 3, 4, 5, 6, 7 |
| Treatment | Fixed | 2 | Diet, Sugar |

**Analysis of Variance**

| Source | DF | Adj SS | Adj MS | F-Value | P-Value |
|---|---|---|---|---|---|
| Subject | 6 | 39886 | 6648 | 2.61 | 0.134 |
| Treatment | 1 | 10314 | 10314 | 4.05 | 0.091 |
| Error | 6 | 15286 | 2548 | | |
| Total | 13 | 65486 | | | |

**Model Summary**

| S | R-sq | R-sq(adj) | R-sq(pred) |
|---|---|---|---|
| 50.4739 | 76.66% | 49.43% | 0.00% |

**Coefficients**

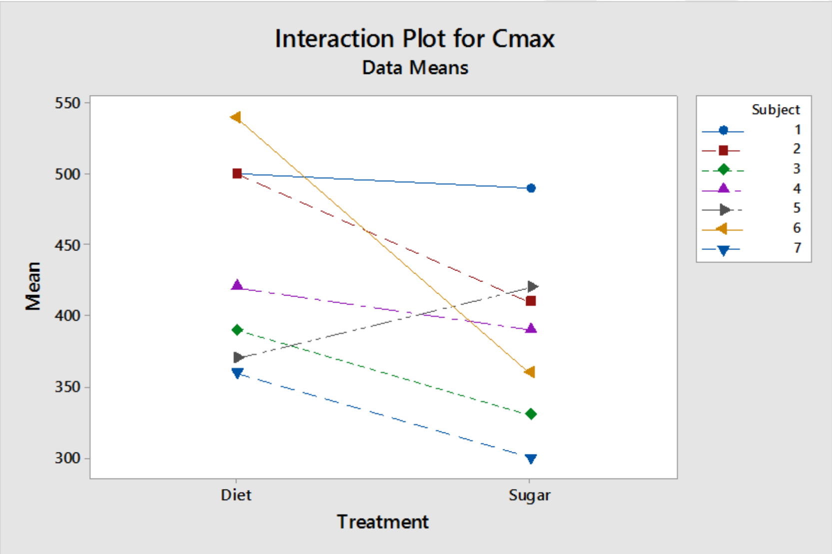
| Term | Coef | SE Coef | T-Value | P-Value | VIF |
|---|---|---|---|---|---|
| Constant | 412.9 | 13.5 | 30.61 | 0.000 | |
| Subject | | | | | |
| 1 | 82.1 | 33.0 | 2.49 | 0.047 | 1.71 |
| 2 | 42.1 | 33.0 | 1.28 | 0.249 | 1.71 |
| 3 | -52.9 | 33.0 | -1.60 | 0.161 | 1.71 |
| 4 | -7.9 | 33.0 | -0.24 | 0.820 | 1.71 |
| 5 | -17.9 | 33.0 | -0.54 | 0.608 | 1.71 |
| 6 | 37.1 | 33.0 | 1.12 | 0.304 | 1.71 |
| Treatment | | | | | |
| Diet | 27.1 | 13.5 | 2.01 | 0.091 | 1.00 |

**Regression Equation**

Cmax = 412.9 + 82.1 Subject_1 + 42.1 Subject_2 - 52.9 Subject_3 - 7.9 Subject_4
- 17.9 Subject_5 + 37.1 Subject_6 - 82.9 Subject_7 + 27.1 Treatment_Diet
- 27.1 Treatment_Sugar

**Means**

| Term | Fitted Mean | SE Mean |
|---|---|---|
| Subject | | |
| 1 | 495.0 | 35.7 |
| 2 | 455.0 | 35.7 |
| 3 | 360.0 | 35.7 |
| 4 | 405.0 | 35.7 |
| 5 | 395.0 | 35.7 |
| 6 | 450.0 | 35.7 |
| 7 | 330.0 | 35.7 |
| Treatment | | |
| Diet | 440.0 | 19.1 |
| Sugar | 385.7 | 19.1 |




**General Linear Model: tmax versus Subject, Treatment**

**Method**

| | |
|---|---|
| Factor coding | (-1, 0, +1) |

**Factor Information**

| Factor | Type | Levels | Values |
|---|---|---|---|
| Subject | Fixed | 7 | 1, 2, 3, 4, 5, 6, 7 |
| Treatment | Fixed | 2 | Diet, Sugar |

**Analysis of Variance**

| Source | DF | Adj SS | Adj MS | F-Value | P-Value |
|---|---|---|---|---|---|
| Subject | 6 | 1285.7 | 214.3 | 1.00 | 0.500 |
| Treatment | 1 | 401.8 | 401.8 | 1.88 | 0.220 |
| Error | 6 | 1285.7 | 214.3 | | |
| Total | 13 | 2973.2 | | | |

**Model Summary**

| S | R-sq | R-sq(adj) | R-sq(pred) |
|---|---|---|---|
| 14.6385 | 56.76% | 6.31% | 0.00% |

**Coefficients**

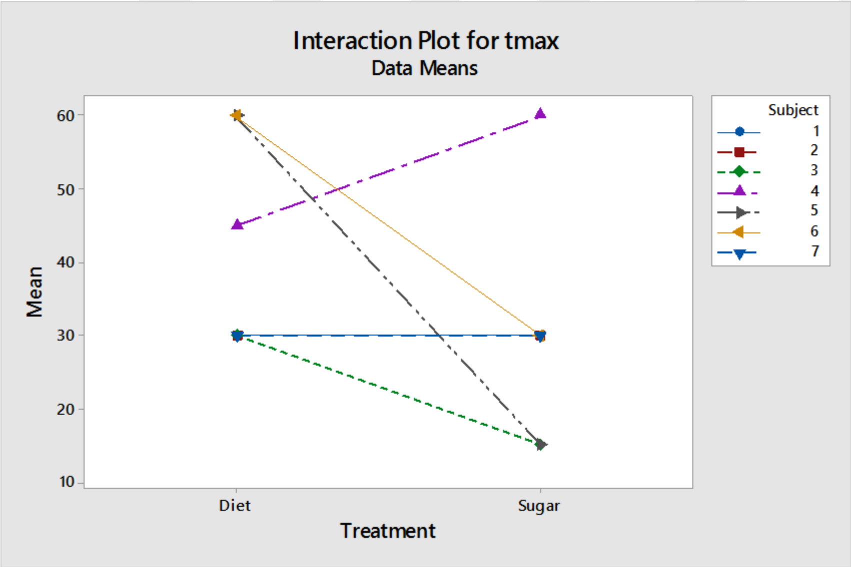
| Term | Coef | SE Coef | T-Value | P-Value | VIF |
|---|---|---|---|---|---|
| Constant | 35.36 | 3.91 | 9.04 | 0.000 | |
| Subject | | | | | |
| 1 | -5.36 | 9.58 | -0.56 | 0.596 | 1.71 |
| 2 | -5.36 | 9.58 | -0.56 | 0.596 | 1.71 |
| 3 | -12.86 | 9.58 | -1.34 | 0.228 | 1.71 |
| 4 | 17.14 | 9.58 | 1.79 | 0.124 | 1.71 |
| 5 | 2.14 | 9.58 | 0.22 | 0.830 | 1.71 |
| 6 | 9.64 | 9.58 | 1.01 | 0.353 | 1.71 |
| Treatment | | | | | |
| Diet | 5.36 | 3.91 | 1.37 | 0.220 | 1.00 |

**Regression Equation**

tmax = 35.36 - 5.36 Subject_1 - 5.36 Subject_2 - 12.86 Subject_3 + 17.14 Subject_4 + 2.14 Subject_5 + 9.64 Subject_6 - 5.36 Subject_7 + 5.36 Treatment_Diet - 5.36 Treatment_Sugar

**Means**

| Term | Fitted Mean | SE Mean |
|---|---|---|
| Subject | | |
| 1 | 30.0 | 10.4 |
| 2 | 30.0 | 10.4 |
| 3 | 22.5 | 10.4 |
| 4 | 52.5 | 10.4 |
| 5 | 37.5 | 10.4 |
| 6 | 45.0 | 10.4 |
| 7 | 30.0 | 10.4 |
| Treatment | | |
| Diet | 40.71 | 5.53 |
| Sugar | 30.00 | 5.53 |



**General Linear Model: Intercept versus Subject, Treatment**

**Method**

| | |
|---|---|
| Factor coding | (-1, 0, +1) |

**Factor Information**

| Factor | Type | Levels | Values |
|---|---|---|---|
| Subject | Fixed | 7 | 1, 2, 3, 4, 5, 6, 7 |
| Treatment | Fixed | 2 | Diet, Sugar |

**Analysis of Variance**

| Source | DF | Adj SS | Adj MS | F-Value | P-Value |
|---|---|---|---|---|---|
| Subject | 6 | 26962 | 4493.6 | 13.91 | 0.003 |
| Treatment | 1 | 6955 | 6955.5 | 21.53 | 0.004 |
| Error | 6 | 1938 | 323.1 | | |
| Total | 13 | 35856 | | | |

**Model Summary**

| S | R-sq | R-sq(adj) | R-sq(pred) |
|---|---|---|---|
| 17.9744 | 94.59% | 88.29% | 70.57% |

**Coefficients**

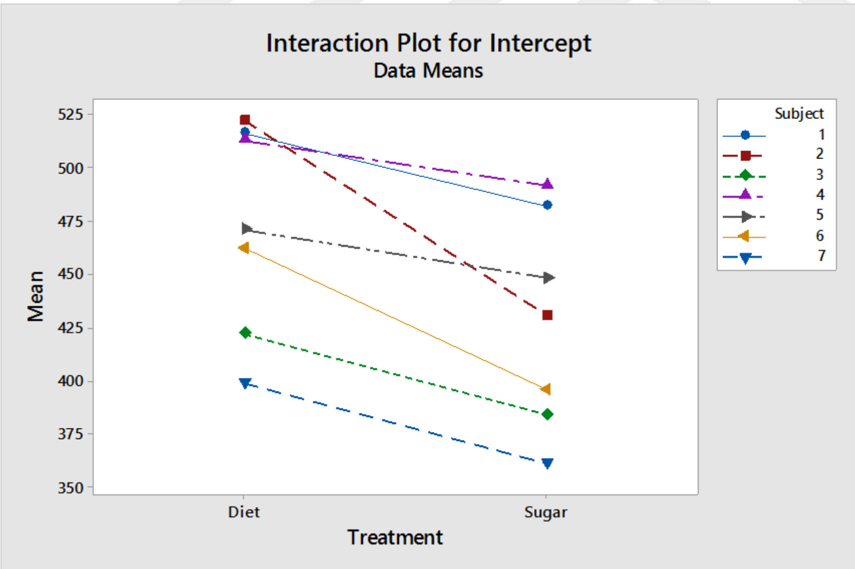
| Term | Coef | SE Coef | T-Value | P-Value | VIF |
|---|---|---|---|---|---|
| Constant | 450.05 | 4.80 | 93.68 | 0.000 | |
| Subject | | | | | |
| 1 | 49.4 | 11.8 | 4.20 | 0.006 | 1.71 |
| 2 | 26.5 | 11.8 | 2.26 | 0.065 | 1.71 |
| 3 | -47.0 | 11.8 | -3.99 | 0.007 | 1.71 |
| 4 | 52.2 | 11.8 | 4.43 | 0.004 | 1.71 |
| 5 | 9.6 | 11.8 | 0.82 | 0.445 | 1.71 |
| 6 | -21.0 | 11.8 | -1.78 | 0.125 | 1.71 |
| Treatment | | | | | |
| Diet | 22.29 | 4.80 | 4.64 | 0.004 | 1.00 |

**Regression Equation**

Intercept = 450.05 + 49.4 Subject_1 + 26.5 Subject_2 - 47.0 Subject_3 + 52.2 Subject_4 + 9.6 Subject_5 - 21.0 Subject_6 - 69.8 Subject_7 + 22.29 Treatment_Diet - 22.29 Treatment_Sugar

**Means**

| Term | Fitted Mean | SE Mean |
|---|---|---|
| Subject | | |
| 1 | 499.5 | 12.7 |
| 2 | 476.6 | 12.7 |
| 3 | 403.1 | 12.7 |
| 4 | 502.2 | 12.7 |
| 5 | 459.7 | 12.7 |
| 6 | 429.1 | 12.7 |
| 7 | 380.2 | 12.7 |
| Treatment | | |
| Diet | 472.33 | 6.79 |
| Sugar | 427.76 | 6.79 |



**General Linear Model: AUC versus Subject, Treatment**

**Method**

| | |
|---|---|
| Factor coding | (-1, 0, +1) |

**Factor Information**

| Factor | Type | Levels | Values |
|---|---|---|---|
| Subject | Fixed | 7 | 1, 2, 3, 4, 5, 6, 7 |
| Treatment | Fixed | 2 | Diet, Sugar |

**Analysis of Variance**

| Source | DF | Adj SS | Adj MS | F-Value | P-Value |
|---|---|---|---|---|---|
| Subject | 6 | 36500 | 6083 | 5.72 | 0.026 |
| Treatment | 1 | 12331 | 12331 | 11.60 | 0.014 |
| Error | 6 | 6376 | 1063 | | |
| Total | 13 | 55207 | | | |

**Model Summary**

| S | R-sq | R-sq(adj) | R-sq(pred) |
|---|---|---|---|
| 32.5990 | 88.45% | 74.98% | 37.12% |

**Coefficients**

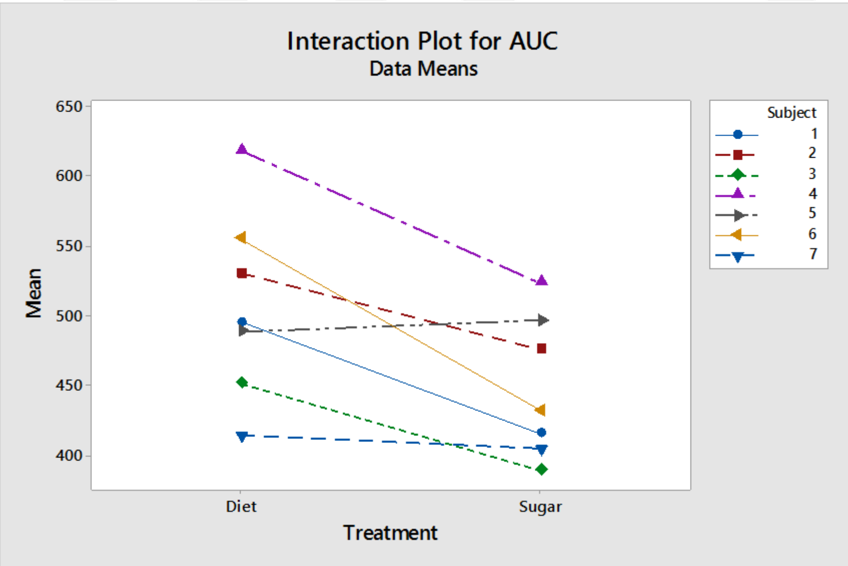
| Term | Coef | SE Coef | T-Value | P-Value | VIF |
|---|---|---|---|---|---|
| Constant | 477.75 | 8.71 | 54.84 | 0.000 | |
| Subject | | | | | |
| 1 | -22.5 | 21.3 | -1.05 | 0.332 | 1.71 |
| 2 | 25.1 | 21.3 | 1.18 | 0.284 | 1.71 |
| 3 | -57.4 | 21.3 | -2.69 | 0.036 | 1.71 |
| 4 | 93.0 | 21.3 | 4.36 | 0.005 | 1.71 |
| 5 | 14.6 | 21.3 | 0.69 | 0.519 | 1.71 |
| 6 | 15.8 | 21.3 | 0.74 | 0.488 | 1.71 |
| Treatment | | | | | |
| Diet | 29.68 | 8.71 | 3.41 | 0.014 | 1.00 |

**Regression Equation**

AUC = 477.75 - 22.5 Subject_1 + 25.1 Subject_2 - 57.4 Subject_3 + 93.0 Subject_4 + 14.6 Subject_5 + 15.8 Subject_6 - 68.6 Subject_7 + 29.68 Treatment_Diet - 29.68 Treatment_Sugar

**Means**

| Term | Fitted Mean | SE Mean |
|---|---|---|
| Subject | | |
| 1 | 455.3 | 23.1 |
| 2 | 502.9 | 23.1 |
| 3 | 420.4 | 23.1 |
| 4 | 570.8 | 23.1 |
| 5 | 492.4 | 23.1 |
| 6 | 493.5 | 23.1 |
| 7 | 409.1 | 23.1 |
| Treatment | | |
| Diet | 507.4 | 12.3 |
| Sugar | 448.1 | 12.3 |



**General Linear Model: Beta versus Subject, Treatment**

**Method**

| | |
|---|---|
| Factor coding | (-1, 0, +1) |

**Factor Information**

| Factor | Type | Levels | Values |
|---|---|---|---|
| Subject | Fixed | 7 | 1, 2, 3, 4, 5, 6, 7 |
| Treatment | Fixed | 2 | Diet, Sugar |

**Analysis of Variance**

| Source | DF | Adj SS | Adj MS | F-Value | P-Value |
|---|---|---|---|---|---|
| Subject | 6 | 10.3615 | 1.7269 | 1.13 | 0.443 |
| Treatment | 1 | 0.2099 | 0.2099 | 0.14 | 0.724 |
| Error | 6 | 9.1778 | 1.5296 | | |
| Total | 13 | 19.7493 | | | |

**Model Summary**

| S | R-sq | R-sq(adj) | R-sq(pred) |
|---|---|---|---|
| 1.23679 | 53.53% | 0.00% | 0.00% |

**Coefficients**

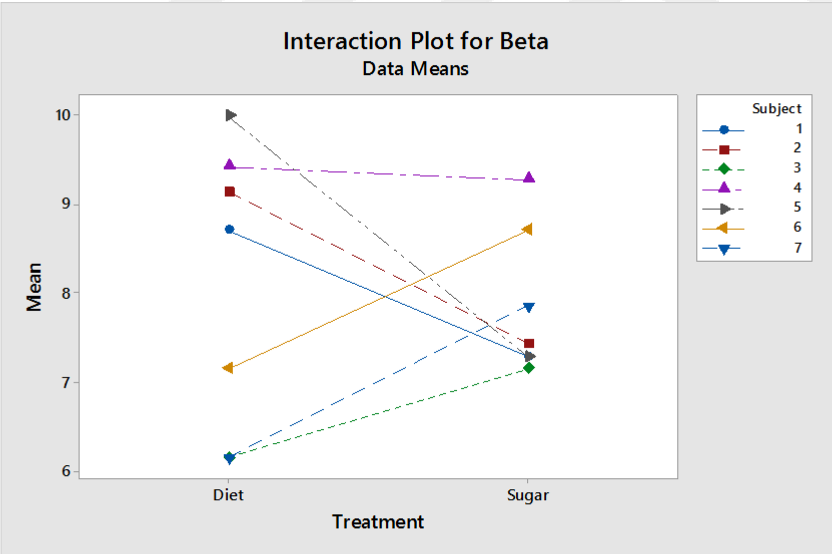
| Term | Coef | SE Coef | T-Value | P-Value | VIF |
|---|---|---|---|---|---|
| Constant | 7.980 | 0.331 | 24.14 | 0.000 | |
| Subject | | | | | |
| 1 | 0.020 | 0.810 | 0.03 | 0.981 | 1.71 |
| 2 | 0.306 | 0.810 | 0.38 | 0.718 | 1.71 |
| 3 | -1.337 | 0.810 | -1.65 | 0.150 | 1.71 |
| 4 | 1.378 | 0.810 | 1.70 | 0.140 | 1.71 |
| 5 | 0.663 | 0.810 | 0.82 | 0.444 | 1.71 |
| 6 | -0.051 | 0.810 | -0.06 | 0.952 | 1.71 |
| Treatment | | | | | |
| Diet | 0.122 | 0.331 | 0.37 | 0.724 | 1.00 |

**Regression Equation**

Beta = 7.980 + 0.020 Subject_1 + 0.306 Subject_2 - 1.337 Subject_3 + 1.378 Subject_4 + 0.663 Subject_5 - 0.051 Subject_6 - 0.980 Subject_7 + 0.122 Treatment_Diet - 0.122 Treatment_Sugar

**Means**

| Term | Fitted Mean | SE Mean |
|---|---|---|
| Subject | | |
| 1 | 8.000 | 0.875 |
| 2 | 8.286 | 0.875 |
| 3 | 6.643 | 0.875 |
| 4 | 9.357 | 0.875 |
| 5 | 8.643 | 0.875 |
| 6 | 7.929 | 0.875 |
| 7 | 7.000 | 0.875 |
| Treatment | | |
| Diet | 8.102 | 0.467 |
| Sugar | 7.857 | 0.467 |



### General Linear Model: Intercept1 versus Subject, Treatment

#### Method

| | |
|---|---|
| Factor coding | (-1, 0, +1) |

#### Factor Information

| Factor | Type | Levels | Values |
|---|---|---|---|
| Subject | Fixed | 7 | 1, 2, 3, 4, 5, 6, 7 |
| Treatment | Fixed | 2 | Diet, Sugar |

#### Analysis of Variance

| Source | DF | Adj SS | Adj MS | F-Value | P-Value |
|---|---|---|---|---|---|
| Subject | 6 | 40983 | 6830.5 | 7.02 | 0.016 |
| Treatment | 1 | 5098 | 5097.5 | 5.24 | 0.062 |
| Error | 6 | 5838 | 972.9 | | |
| Total | 13 | 51918 | | | |

#### Model Summary

| S | R-sq | R-sq(adj) | R-sq(pred) |
|---|---|---|---|
| 31.1921 | 88.76% | 75.64% | 38.78% |

#### Coefficients

| Term | Coef | SE Coef | T-Value | P-Value | VIF |
|---|---|---|---|---|---|
| Constant | 394.85 | 8.34 | 47.36 | 0.000 | |
| Subject | | | | | |
| 1 | -47.0 | 20.4 | -2.30 | 0.061 | 1.71 |
| 2 | 4.4 | 20.4 | 0.22 | 0.835 | 1.71 |
| 3 | -61.1 | 20.4 | -2.99 | 0.024 | 1.71 |
| 4 | 107.8 | 20.4 | 5.28 | 0.002 | 1.71 |
| 5 | 17.5 | 20.4 | 0.86 | 0.425 | 1.71 |
| 6 | 23.5 | 20.4 | 1.15 | 0.293 | 1.71 |
| Treatment | | | | | |
| Diet | 19.08 | 8.34 | 2.29 | 0.062 | 1.00 |

**Regression Equation**

Intercept1 = 394.85 - 47.0 Subject_1 + 4.4 Subject_2 - 61.1 Subject_3 + 107.8 Subject_4 + 17.5 Subject_5 + 23.5 Subject_6 - 45.2 Subject_7 + 19.08 Treatment_Diet - 19.08 Treatment_Sugar

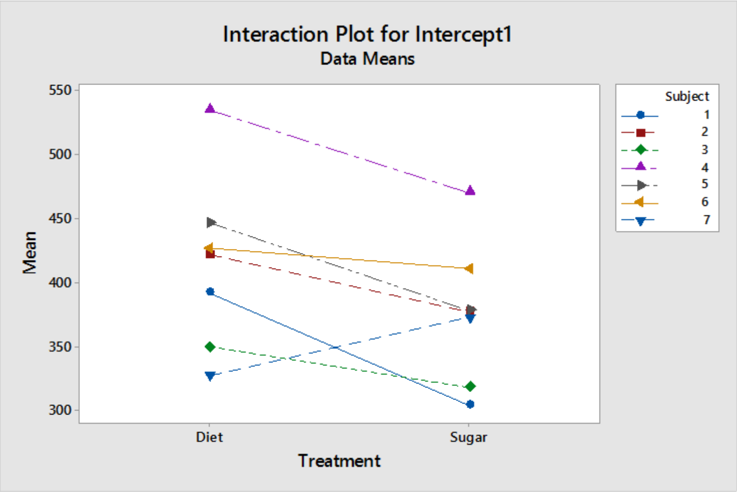
**Fits and Diagnostics for Unusual Observations**

| Obs | Intercept1 | Fit | Resid | Std Resid | |
|---|---|---|---|---|---|
| 13 | 372.5 | 330.6 | 41.9 | 2.05 | R |
| 14 | 326.8 | 368.7 | -41.9 | -2.05 | R |

*R Large residual*

**Means**

| Term | Fitted Mean | SE Mean |
|---|---|---|
| Subject | | |
| 1 | 347.9 | 22.1 |
| 2 | 399.3 | 22.1 |
| 3 | 333.8 | 22.1 |
| 4 | 502.7 | 22.1 |
| 5 | 412.3 | 22.1 |
| 6 | 418.4 | 22.1 |
| 7 | 349.6 | 22.1 |
| Treatment | | |
| Diet | 413.9 | 11.8 |
| Sugar | 375.8 | 11.8 |



**General Linear Model: t½ versus Subject, Treatment**

**Method**

| | |
|---|---|
| Factor coding | (-1, 0, +1) |

**Factor Information**

| Factor | Type | Levels | Values |
|---|---|---|---|
| Subject | Fixed | 7 | 1, 2, 3, 4, 5, 6, 7 |
| Treatment | Fixed | 2 | Diet, Sugar |

**Analysis of Variance**

| Source | DF | Adj SS | Adj MS | F-Value | P-Value |
|---|---|---|---|---|---|
| Subject | 6 | 1786.85 | 297.81 | 5.44 | 0.029 |
| Treatment | 1 | 28.57 | 28.57 | 0.52 | 0.497 |
| Error | 6 | 328.19 | 54.70 | | |
| Total | 13 | 2143.61 | | | |

**Model Summary**

| S | R-sq | R-sq(adj) | R-sq(pred) |
|---|---|---|---|
| 7.39582 | 84.69% | 66.83% | 16.65% |

**Coefficients**

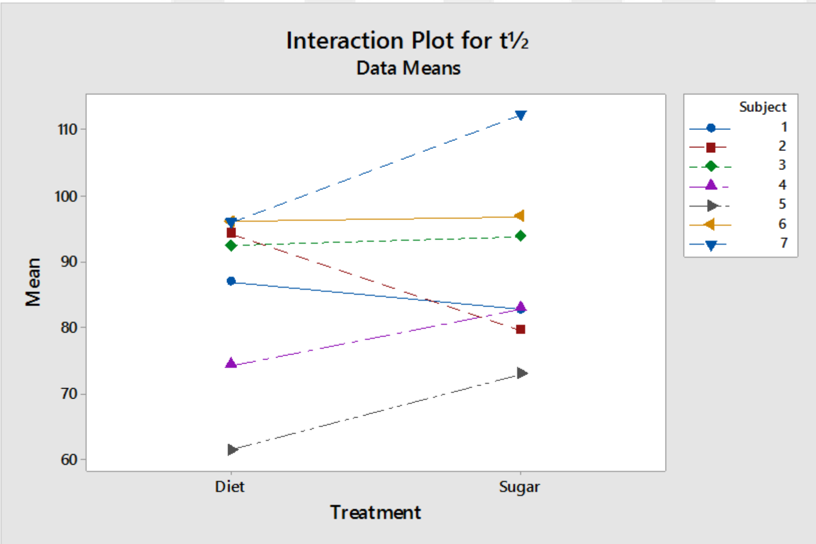
| Term | Coef | SE Coef | T-Value | P-Value | VIF |
|---|---|---|---|---|---|
| Constant | 87.33 | 1.98 | 44.18 | 0.000 | |
| Subject | | | | | |
| 1 | -2.53 | 4.84 | -0.52 | 0.620 | 1.71 |
| 2 | -0.38 | 4.84 | -0.08 | 0.940 | 1.71 |
| 3 | 5.82 | 4.84 | 1.20 | 0.275 | 1.71 |
| 4 | -8.78 | 4.84 | -1.81 | 0.120 | 1.71 |
| 5 | -20.18 | 4.84 | -4.17 | 0.006 | 1.71 |
| 6 | 9.17 | 4.84 | 1.89 | 0.107 | 1.71 |
| Treatment | | | | | |
| Diet | -1.43 | 1.98 | -0.72 | 0.497 | 1.00 |

**Regression Equation**

t½ = 87.33 - 2.53 Subject_1 - 0.38 Subject_2 + 5.82 Subject_3 - 8.78 Subject_4 - 20.18 Subject_5 + 9.17 Subject_6 + 16.87 Subject_7 - 1.43 Treatment_Diet + 1.43 Treatment_Sugar

**Means**

| Term | Fitted Mean | SE Mean |
|---|---|---|
| Subject | | |
| 1 | 84.80 | 5.23 |
| 2 | 86.95 | 5.23 |
| 3 | 93.15 | 5.23 |
| 4 | 78.55 | 5.23 |
| 5 | 67.15 | 5.23 |
| 6 | 96.50 | 5.23 |
| 7 | 104.20 | 5.23 |
| Treatment | | |
| Diet | 85.90 | 2.80 |
| Sugar | 88.76 | 2.80 |



**General Linear Model: Tlag versus Subject, Treatment**

**Method**

| | |
|---|---|
| Factor coding | (-1, 0, +1) |

**Factor Information**

| Factor | Type | Levels | Values |
|---|---|---|---|
| Subject | Fixed | 7 | 1, 2, 3, 4, 5, 6, 7 |
| Treatment | Fixed | 2 | Diet, Sugar |

**Analysis of Variance**

| Source | DF | Adj SS | Adj MS | F-Value | P-Value |
|---|---|---|---|---|---|
| Subject | 6 | 1089.6 | 181.6 | 1.36 | 0.359 |
| Treatment | 1 | 157.1 | 157.1 | 1.18 | 0.320 |
| Error | 6 | 800.7 | 133.5 | | |
| Total | 13 | 2047.4 | | | |

**Model Summary**

| S | R-sq | R-sq(adj) | R-sq(pred) |
|---|---|---|---|
| 11.5521 | 60.89% | 15.27% | 0.00% |

**Coefficients**

| Term | Coef | SE Coef | T-Value | P-Value | VIF |
|---|---|---|---|---|---|
| Constant | 47.09 | 3.09 | 15.25 | 0.000 | |
| Subject | | | | | |
| 1 | -7.49 | 7.56 | -0.99 | 0.360 | 1.71 |
| 2 | 2.51 | 7.56 | 0.33 | 0.752 | 1.71 |
| 3 | 8.66 | 7.56 | 1.14 | 0.296 | 1.71 |
| 4 | -3.44 | 7.56 | -0.46 | 0.665 | 1.71 |
| 5 | -13.14 | 7.56 | -1.74 | 0.133 | 1.71 |
| 6 | -1.89 | 7.56 | -0.25 | 0.811 | 1.71 |
| Treatment | | | | | |
| Diet | -3.35 | 3.09 | -1.09 | 0.320 | 1.00 |

**Regression Equation**

Tlag = 47.09 - 7.49 Subject_1 + 2.51 Subject_2 + 8.66 Subject_3 - 3.44 Subject_4 - 13.14 Subject_5 - 1.89 Subject_6 + 14.81 Subject_7 - 3.35 Treatment_Diet + 3.35 Treatment_Sugar

**Means**

| Term | Fitted Mean | SE Mean |
|---|---|---|
| Subject | | |
| 1 | 39.60 | 8.17 |
| 2 | 49.60 | 8.17 |
| 3 | 55.75 | 8.17 |
| 4 | 43.65 | 8.17 |
| 5 | 33.95 | 8.17 |
| 6 | 45.20 | 8.17 |
| 7 | 61.90 | 8.17 |
| Treatment | | |
| Diet | 43.74 | 4.37 |
| Sugar | 50.44 | 4.37 |