Effects of Replacing Diet Beverages with Water on Weight loss, Carbohydrate and Lipid Metabolism during a Hypoenergetic Diet

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Content

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ABSTRACT

This thesis compared the effect on weight loss of either replacing diet beverages (DBs) with water or continuing to consume DBs (study 1 in healthy overweight and obese, n=89 and study 2 in patients with type 2 diabetes, n=81) adults during a 24-wk weight-loss program (for study1 and 2) and 18 month weight loss and weight maintenance plan (for study3, n=89).

In study 1, compared with the DB group, the water group had a greater decrease in weight (1.2kg more weight loss in the water group compared with the DBs Group ,P=0.015), BMI (0.5 kg/m² more BMI reduction in the water compared with the DBs Group ,P=0.002), fasting insulin (1 mU/l more Fasting insulin reduction in the water compared with the DBs Group,P<0.001), homeostasis model assessment of insulin resistance (HOMA-IR)(0.2 more HOMA-IR reduction in the water compared with the DBs Group, P<0.001) and 2-h postprandial glucose(0.305 mmol/l more 2hpp reduction in the water compared with the DBs Group,P<0.001).

In study 2, weight (2.5 kg more weight loss in the water compared with the DBs Group, P=0.005) and BMI (0.9 kg/m² more BMI reduction in the water group compared with the DBs Group, P=0.005) decrease after 18 months in the water group compared with the DBs group was significant. There was also a greater reduction in fasting insulin levels (1.8 mU/l more Fasting insulin reduction in the water group compared with the DBs Group,P<0.001), better improvement in HOMA-IR (0.5 more HOMA-IR reduction in the water group compared with the DBs Group, P<0.001), a greater decrease in 2-h postprandial plasma glucose (0.5 mmol/l more 2hpp reduction in the water group

compared with the DBs Group, $P<0.001$) and glycated hemoglobin (0.2 more HbA1c reduction in the water group compared with the DBs Group, $P=0.030$) in the water group compared with the DBs over 18 months.

In study 3, obese participants with Type 2 diabetes in the water group of study 2 had more weight loss (1.16 kg more weight loss in the water group compared with the DBs Group, $P=0.006$) and BMI (0.5 kg/m^2 more BMI reduction in the water group compared with the DBs Group, $P=0.006$). Also reduction of fasting insulin (1.6 mU/l more Fasting insulin reduction in water group compared with DBs Group, $P<0.001$), fasting plasma glucose (0.3mmol/l more Fasting plasma glucose reduction in the water group compared with the DBs Group, $P=0.003$), HOMA-IR (0.7 more HOMA-IR reduction in the water group compared with the DBs Group, $P=0.003$) and 2 hour postprandial glucose (0.3 mmol/l more 2hpp reduction in the water group compared with the DBs Group, $P=0.027$) was greater in the water group.

By contrast, changes in waist circumference and lipid profiles were not significantly different between the two groups in these three studies.

Replacement of DBs with water after the main meal in obese and overweight healthy and type 2 diabetic women who were regular users of DBs may cause greater weight reduction during 24 weeks and also in the longer period of an 18 month weight management program. In addition, replacement of DBs with water offers clinical benefits to improve insulin resistance. Generally, better improvements in energy and carbohydrate metabolism may occur when water rather than DBs is consumed over a weight loss program.

PUBLICATIONS

The work described in this thesis has been disseminated previously in the following forms:

1. PAPERS:

A. Ameneh Madjd, Moira A Taylor, Alireza Delavari, Reza Malekzadeh, Ian A Macdonald, and Hamid R Farshchi
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Am J Clin Nutr. 2015 Dec; 102(6):1305-12. doi: 10.3945/ajcn.115.109397

B. Ameneh Madjd, Moira A Taylor, Alireza Delavari, Reza Malekzadeh, Ian A Macdonald, and Hamid R Farshchi
Beneficial effects of replacing diet beverages with water on type 2 diabetic obese women following a hypo-energetic diet: A randomized, 24-week clinical trial.
Diabetes Obes Metab. 2016 Oct 16. doi: 10.1111/dom.12793

C. Ameneh Madjd, Moira A Taylor, Alireza Delavari, Reza Malekzadeh, Ian A Macdonald, and Hamid R Farshchi.
Effects of replacing diet beverages with water on weight loss and weight maintenance: 18 month follow up, randomized clinical trial, Submitted in IJO, Jan 2017

2) ABSTRACTS

I. Ameneh Madjd, Moira A Taylor, Hamid R Farshchi and Ian A Macdonald
Effects on weight loss in adults of replacing diet beverages with water during a hypoenergetic diet: a randomized, 24

week clinical trial. Life Sciences PGR Symposium, June 2015, Nottingham, UK. (Poster presentation).

II. Ameneh Madjd
Effects of replacing diet beverages with water on weight loss in adults during a hypo-energetic diet. ASO UK Congress on Obesity, September 2015, Glasgow, UK (oral presentation).

III. Ameneh Madjd, Moira A Taylor, Hamid R Farshchi and Ian A Macdonald
Beneficial effects of replacing diet beverages with water on Type 2 diabetic obese women following a hypo-energetic diet - a randomized, 24 week clinical trial. UKCO2016, September 2016, Nottingham, UK. (Poster presentation).

Declaration

I hereby declare that the data for present thesis has been collected by myself and the work which has been mentioned was performed by myself, except where assistance has been acknowledged .No part of this thesis has been submitted in any previous application for higher degree.

Ameneh Madjd

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of energy restriction with either drinking water

or diet beverages in all participants.

ABBREVIATIONS

- Analysis of variance: ANOVA
- Cognitive behavioural therapy: CBT
- Diet beverages: DBs
- Fasting plasma glucose: FPG
- Glycated hemoglobin: Hb A_{1C}
- Homeostasis model assessment of insulin resistance: HOMA-IR
- Hour: h
- Intestinal alkaline phosphatase: IAP
- Liter: l
- Mole: mol
- Non-nutritive sweetener: NNS
- Phenylalanine: PHE
- Standard deviation: SD
- Sugar sweetened beverages: SSBs
- Total cholesterol: TC
- Triglyceride: TG
- 2 hour post prandial: 2hpp
- Waist circumference: WC

Chapter 1.General Introduction

1.1 Introduction

Obesity has become one of the most vital public health problems in the United States and many other resource-rich countries and transitional economies (1, 2). In fact, obesity is a chronic disease that is increasing in prevalence in adults, adolescents and children and is now considered to be a global epidemic. Obesity can also increase the risk of diabetes (3), cardiovascular diseases (4, 5) and hypertension (6, 7).

Obesity and its related disorders are linked to certain dietary choices that are considered unhealthy. It seems that people are moving away from having a healthy diet (8) and increasingly consume more energy dense foods. In the past decades, consumption of energy containing beverages appears to have risen around the world (9). Obese people believe that drinking diet beverages (DBs) may be a simple strategy to achieve weight loss. However, usually nutritionists advise obese people to drink water when attempting to lose weight.

To my knowledge, there is no study comparing the effects of replacing diet beverages with water on weight loss management and biochemical cardiovascular risk factors in obese people following a comprehensive weight loss diet program. The following literature review starts with a general introduction to obesity and its physiological aspects and then considers some of the literature which is concerned with consumption of sugar sweetened beverages and its replacement with diet beverages and water. It then attempts to consider obesity management methods and also type2 diabetes and its management. At the end, the proposed hypotheses and topics of this thesis are described.

1.2 Obesity: definition and its importance for health

Obesity is defined as a condition of excess stores of body fat which impairs health (3). The National Heart, Lung, and Blood Institute (10) and the World Health Organization (11, 12) have provided the same definitions of overweight and obesity. Although "overweight" technically relates to an excess of body weight and "obesity" to an excess of fat. Methods used to assess body fat directly are not available in daily practice. For this reason, obesity is often measured by means of indirect estimates of body fat (13). The body mass index (11) is the accepted standard measure of obesity (14) and is equal to the body weight divided by the height squared.

- ✓ In most but not all ethnic groups,a BMI which exceeds 30 kg/m^2 is considered as *obese* (11)
  - A BMI ≥ 35 or 40 kg/m^2 is *severe obesity*
  - A BMI of ≥ 35 kg/m^2 concurrently with obesity-related health conditions or ≥40–44.9 kg/m^2 is *morbid obesity*
  - A BMI of ≥ 45 or 50 kg/m^2 is *super obesity*
- ✓ The WHO and the National Heart, Lung, and Blood Institute also define overweight as a BMI >25 and ≤29.9 kg/m^2.

Obesity is now recognized as a major determinant of many non-communicable diseases including cardiovascular diseases (4, 5), diabetes (3), hyperlipidaemia (15) and mental health problems (16) which are associated with morbidity, and is associated with increased mortality due to coronary heart disease(4), cancer (17) and diabetes mellitus (18).

One important category of obesity not captured by BMI is so-called "abdominal obesity"— also known as central obesity. This exists when excessive abdominal fat around the stomach and abdomen has built up to the extent that it is likely to have a negative impact on health. There is a strong correlation between central obesity and cardiovascular diseases (19). Furthermore, abdominal obesity has been linked to Alzheimer's disease (20) and type 2 diabetes (21).

The simplest and most often used measure of abdominal obesity is waist size. Guidelines generally define abdominal obesity in women as a waist size 89cm or higher, and in men as a waist size of 102cm or higher.

The increasing prevalence of obesity and its major deleterious effects on human health, mean that identification of successful methods for obesity prevention and treatment are necessary to reduce individual suffering and economic cost.

1.2.1 Prevalence of obesity

The prevalence of obesity considerably differs between countries and between regions within countries. In many countries, overweight is more common among men than women, but obesity is more common in women (22).

In 2008, 35% of adults aged 20+ were overweight (BMI ≥ 25 kg/m^2) (34% men and 35% of women) in 199 countries and territories (23). The worldwide prevalence of obesity has nearly doubled between 1980 and 2008. In 2008, 10% of men and 14% of women in the world were obese (BMI ≥30 kg/m^2), compared with 5% for men and 8% for women in 1980. Estimated long-term risks for overweight or obesity topped exceeded 50% and 25%, respectively, demonstrating a large public health burden worldwide (24).

The MONICA Study (1989) showed that the average prevalence of obesity was about 15% in men and 22% in women in European countries (25). In Europe, the prevalence of obesity in men ranged from 4.0% to 28.3% and in women from 6.2% to 36.5% with geographical differences between countries (26). National surveys in the USA have also demonstrated relatively rapid increases in prevalence of overweight (27). The prevalence of obesity has been also increased during the last decades in developing countries, where obesity is emerging as a major health problem in common with the western world. In 2008, in Semnan in Iran the prevalence of obesity was 26.3%, with a higher prevalence in women (39.5%) than men (14.5%) (28).

The longitudinal Framingham Heart Study illustrated that the long-term risk of becoming overweight (BMI ≥25 kg/m²) or obese (BMI ≥30 kg/m²) during adulthood appears to be very high. From 4117 men and women (mostly Caucasian, ages 30 to 59 years) the following results were observed from 1971-2001(24):

1. Within four years, 14 to 19 percent of women and 26 to 30 percent of men who were normal weight at baseline became overweight. Also 5 to 9 percent of men and women who were either normal weight or overweight at baseline became obese.
2. During 30 years, more than 50 percent of individuals became overweight, while about 30 percent of women and 25 percent and men became obese.

This paper was published in 2005 and the current situation may be even worse.

This study indicated that the prevalence of overweight and obesity were highest in the WHO Regions of the Americas (62% for overweight in both sexes, and 26% for obesity) and lowest in the WHO Region for South East Asia (14% overweight in both sexes and 3% for obesity). In the WHO Regions for Europe, the Eastern Mediterranean and the Americas over 50% of women were overweight. For all three of these regions, roughly half of overweight women are obese (23% in Europe, 24% in the Eastern Mediterranean, 29% in the Americas). In all WHO regions women were more likely to be obese than men. In the WHO regions for Africa, Eastern Mediterranean and South East Asia, the prevalence of obesity was about double in women compared with men (24).

The overall prevalence of obesity in Iran increased from 13.6% in 1999 to 19.6% in 2005 and 22.3% in 2007. For overweight subjects, the rates were, respectively, 32.2%, 35.8% and 36.3%. During these years, the mean body mass index (BMI) (kg/m^2) increased from 25.03 in 1999, to 26.14 in 2005, and 26.47 in 2007. The increase in prevalence of obesity was seen in both males and females and both urban and rural residents (29).

A study (30) published in 2010 showed that the prevalence of overweight or obesity (body mass index $\geq 25\,kg/m^2$) was 49.7% in men and 63.9% in women in Shiraz (Southern Iran). The prevalence of obesity (body mass index $\geq 30\,kg/m^2$) was 10.5% and 22.5% in men and women, respectively, which shows an increased secular change of 5.8% in men and 17.4% in women during a 14-year period. This study also indicated that obesity and overweight were significantly more common among women than among men (30).

1.2.2 Cause of obesity

In spite of a widespread desire to control body weight, the prevalence of obesity continues to rise all over the world. The energy balance equation (Δ body energy store=energy intake-energy expenditure) demonstrates that an accumulation of body fat will only occur when energy balance is positive. In other words, body weight (or more precisely body energy content) can only increase when energy intake exceeds energy expenditure for sufficient time for a change in body energy stores to occur. Therefore, the aim of obesity treatment is to induce negative energy balance and mobilize body fat store.

A combination of excess extreme food energy intake and lack of physical activity is considered to explain most cases of obesity (31). A limited number of cases is due to genetics, medical reasons, or psychiatric illness (32, 33). A review identified several other possible contributors to the recent increase of obesity: (1) insufficient sleep, (2) endocrine disruptors (environmental pollutants that interfere with lipid metabolism), (3) reduced variability in ambient temperature, (4) decreased rates of smoking, as smoking suppresses appetite(34), (5) increased use of medications that can cause weight gain (e.g., atypical antipsychotics), (6) proportional increases in ethnic and age groups that tend to be heavier, (7) pregnancy at a later age (which may cause susceptibility to obesity in children) (35). While there is considerable evidence supporting the effect of these mechanisms on the increased prevalence of obesity, the evidence is still inconclusive, and the authors state that these are probably less influential than the ones discussed earlier.

However the underlying cause remains an imbalance between energy intake and energy expenditure. Rising rates of obesity at a societal level could be due to an increase in easily accessible and tasty foods (36, 37), increased sedentary behavior due to the use of cars and mechanized manufacturing which reduces occupational energy expenditure(38, 39). In spite of less physical activity, as a component of energy expenditure, obese people, on average, have a greater energy expenditure than their healthy weight counterparts due to the energy required to maintain their greater body mass (3). So, evidence does not support the commonly expressed view that some obese people eat little yet gain weight due to a slow metabolism. On average obese people have greater total energy expenditure than normal or underweight people and actually have a higher basal metabolic rate per person (BMR) (40).

Diet plays an important role in the beginning of obesity. Higher consumption of energy increased the chances of obesity (41). Per capita, dietary energy supply varies significantly between different regions and countries, and this has changed markedly over time (42).

Rates of obesity in the United States increased from 14.5% to 30.9%, from 1971 to 2000 (43) and during the same period, a rise occurred in the average amount of food energy consumed. The average rise was 335 kcal per day in women (1,542 kcal in 1971 and 1,877 kcal in 2004), while the average rise was 168 kcal per day for men (2,450 kcal in 1971 and 2,618 kcal in 2004). Most of this extra food energy came from a rise in consumption of carbohydrate rather than fat consumption (44). It has been shown that the primary sources of these extra carbohydrates are sweetened beverages, which now in America account for almost 25 percent of daily food energy in young adults (45), and then potato chips (46). It is believed that consumption of sweetened drinks contributes to the rising rates of obesity (47, 48).

A study published in 2010 showed that major dietary patterns of people in Iran had a significant association with general and central obesity (49). Another study also demonstrated that central adiposity is associated with poor lifestyle factors including low physical activity, depression, smoking, low intake of vitamin C, low intake of calcium and dairy products and high fat consumption among females in Tehran (50).

Furthermore, a review by Malekzadeh et al. indicated that the increased consumption of energy-dense regular and fast foods and sucrose-enriched drinks, together with an increasingly sedentary lifestyle, appear to be major factors contributing to the epidemic of obesity in Iran. The per capita consumption of carbonated beverages in Iran is 42 litres per year and 40% of Iranians consume more food than they need, and the average Iranian consumes 40% more carbohydrate and 30% more fat than their needs (51).

1.2.3 Obesity's effect on health

The large numbers of epidemiological studies have evaluated the relationship between obesity and mortality (52, 53). Generally, greater BMI is associated with increased rate of death from cardiovascular disease (CVD) and all other causes. This is specifically true for those with intense obesity (54). Estimation for the annual number of excess deaths ascribable to obesity in the US is fluctuating and changes from 111,909 to 365,000 (33, 36).

As a result of cardiovascular and other non- communicable diseases following obesity, obesity could reduce life expectancy. BMI of 30–35 kg/m^2 decreases life expectancy by two to four years (55), whilst severe obesity (BMI > 40 kg/m^2) decreases life expectancy by ten years (55). On average, obesity decreases life expectancy by six to seven years (3, 34).

Obesity is one of the leading preventable causes of death worldwide (56). It also increases the risk of many physical and mental conditions (57, 58). Complications associated with obesity are either directly caused by obesity or indirectly related via mechanisms sharing a common cause such as a poor diet or a sedentary lifestyle. The strength of the link between obesity and specific conditions varies. Co-morbidities are most commonly shown when combinations of medical disorders are present, labelled by some as the metabolic syndrome. The cluster includes: diabetes, high blood pressure, hypercholesterolamia, abdominal obesity and hypertriglyceridamia (59). One of the strongest links between obesity and disease is the connection with type 2 diabetes. Excess body fat underlies 64% of cases of type 2 diabetes in men and 77% of cases in women (60).

The negative consequences to health of abdominal obesity are even greater than total obesity. Abdominal obesity which clinically known as central obesity, is when excessive abdominal fat around the stomach and abdomen has built up to the extent, has been linked to Alzheimer's Disease in addition to other metabolic and vascular diseases (20). Central obesity is also associated with a statistically higher risk of heart disease, hypertension, insulin resistance, and type 2 diabetes mellitus (61). So, it is now generally believed that intra-abdominal fat is the depot that conveys the biggest health risk (62).

Increased central fat and obesity are also associated with greater morbidity in addition to mortality (3). In a study in the United States, overweight and obese individuals had a higher comparative risk of hypertension, hypercholesterolemia, and diabetes mellitus, compared with normal weight individuals (63).

Epidemiological studies showed that the prevalence of obesity, overweight, and metabolic syndrome in Iran is equal to or higher than in Europe and the United States and it is associated with rising prevalence of Type 2 diabetes and the important comorbid status such as hypertension, cardiovascular, renal, and gastrointestinal diseases along with increasing the burden of cancers. This is also in line with the present etiologies of death in Iran with cardiovascular disease and cancer accounting for nearly 60% of causes of nondramatic death (51).

1.2.4 Obesity and energy intake

Obesity generally happens when energy intake is in excess of energy requirement. A meal is an eating occasion that takes place at a certain time and includes specific, prepared food, or the food eaten on that occasion. Energy intake is calories consumed as food and drink, which is firm by hunger (hypothalamus) and choice (cerebral cortex). Energy intake differs according to the number and/or volume of meals eaten as well as the meal composition. Appetite is stimulated by general hunger and specific food craving which lead to both a general rise in eating behavior and selection of specific foods. Internal signals such as the dynamics of blood glucose concentration may establish a consistent stimulus for the start of eating (64). There are many physiological mechanisms that control starting and stopping a meal. The control of food intake is a physiologically complex, motivated behavioral system.

Drinks and beverages as a component of food intake may also affect the whole daily energy intake and metabolism, and consequently weight control.

1.3 Beverages & weight

1.3.1 Definition of beverage

In definition, beverage is a kind of liquid which is specifically prepared for human consumption. They can be divided into various groups such as plain water, alcohol, non-alcoholic drinks, soft drinks (carbonated drinks), fruit or vegetable juices and hot drinks, such as hot chocolate.

Water has always been an important and life-sustaining drink and is essential for human survival. Water composes approximately 70% by mass of the fat-free component of the human body. It is a crucial component of metabolic processes and function as a solvent for many important solutes.

The term "soft drink" specifies the absence of alcohol in contrast to a "hard drink" or "drink". Small amounts of alcohol may be present in a soft drink, but if the drink is to be considered non-alcoholic the alcohol content must be less than 0.5% of the total volume (65). Beverages such as soda , sparkling water, iced tea, lemonade, root beer, fruit punch, milk, hot chocolate, tea, coffee, milkshakes, and tap water and energy drinks are all soft drinks. Many commercially produced soft drinks typically contain water (often, but not always, carbonated), usually with a sweetener and/or a flavoring agent. The sweetener may be sugar, high-fructose corn syrup, fruit juice (as in sugar sweetened beverages), sugar substitutes (in the case of diet drinks) or some combination of these. Soft drinks may also contain caffeine, colorings, preservatives and other ingredients.

1.3.2 Types of diet beverages

Diet beverages (alternatively marketed as sugar-free or light) are typically sugar-free and artificially sweetened and alcohol free. For the purpose of this thesis they will be considered to be nonalcoholic, hence a soft drink. They are generally marketed toward health-conscious people, diabetics, athletes, and other people who want to lose weight, improve physical fitness, or reduce their sugar intake.

Multiple artificial sweeteners can be used to give a ‘soft drink’ a sweet taste without the sugar. Sometimes two sweeteners are used in the same product. Aspartame is one of the most commonly used artificial sweeteners. Today, at least in the United States, "diet" is nearly synonymous with the use of aspartame in beverages. One of the first artificial sweeteners used in diet beverages were cyclamates (often synergistically with saccharin). In 1970, the Food and Drug Administration (FDA) banned cyclamates in the United States on evidence that they caused cancer in lab rats. Cyclamates are still used in many countries outside of the United States. Once cyclamates were banned, American producers turned back to saccharin. In 1977, the FDA was appealed to ban saccharin, too, as a carcinogen, but a moratorium was placed on the ban until studies were conducted. The ban was lifted in 1991, but by that time, virtually all diet soft drink production had shifted to using aspartame.

Recently, two other sweeteners have been used with increasing frequency: sucralose (marketed as Splenda) and acesulfame potassium ("Sunett" or "Ace K"). The K in "Ace K" represents the chemical symbol for potassium. Acesulfame potassium is usually combined with aspartame, sucralose, or saccharin rather than alone and its use is particularly common among smaller beverage producers (e.g. Big Red). Diet Rite is the non-aspartame diet soda brand with the highest sales today; it uses a combination of sucralose and acesulfame potassium.

In 2005, the Coca-Cola Company announced it would produce a sucralose-containing formulation of diet drink known as Diet Coke with Splenda, but that it would continue to produce the aspartame version as well. There were also rumors that a sugar-free version of Coca-Cola Classic, also sweetened with sucralose, was being formulated as well. This formulation was eventually called Coca-Cola Zero, though it is sweetened with aspartame in conjunction with acesulfame potassium.

Aspartame is a low-calorie, intense artificial sweetener. Aspartame and its breakdown products have been a matter of extensive study for more than 30 years. It has been found to be safe and authorized for human consumption for many years and in many countries following thorough safety assessments (www.efsa.europa.eu/en/topics/topic/aspartame).

In 2011, EFSA's NDA Panel evaluated the confirmation of claims related to intense sweeteners and certain future beneficial health effects. The Panel determined that there is sufficient scientific information to support the claims that intense sweeteners as all sugar replacers lead to a lower rise in blood sugar levels after meals, if consumed instead of sugars, and maintain tooth mineralisation by decreasing tooth demineralisation again if consumed instead of sugars. However, EFSA's experts could find no clear cause and effect relationship to substantiate the claims that intense sweeteners when replacing sugars maintain normal blood sugar levels, or maintain/ achieve a normal body weight.

The amounts and types of artificial sweeteners in diet beverages are different. The table below (Table 1.1) displays the quantity of different sweeteners in a 237ml serving of bottled or canned soda as provided by the manufacturers. To determine the amount in a 355ml can of soft drink, multiply these numbers by 1.5.

Table 1.1 The amounts and types of artificial sweeteners in diet beverages

Soda	SACCHARIN(mg)	ASPARTAME(mg)	ACESULFAME(mg)	SUCRALOSE(mg)
Diet Coke	0	125	0	0
Diet Coke, caffeine free	0	125	0	0
Diet Coke with splenda	0	0	30	40
Coke Zero	0	58	31	0
Diet Pepsi	0	111	7	0
Caffeine Free Diet Pepsi	0	118	0	0

1.3.3 Sugar-sweetened beverages and obesity

About half of total added sugar consumed in the United States is in the form of liquid (66). The greatest consumption of sugar sweetened beverages is in teenage boys, where over 357 kcal per day is consumed (66).

Evidence from large-scale cross-sectional and prospective cohort studies supports that in the United States population consumption of sugar-sweetened beverages (SSBs) is associated with epidemic of obesity in adults and children (67-71). SSBs are beverages that contain added energy containing sweeteners such as sucrose, high-fructose corn syrup or fruit-juice concentrates, all of which result in similar metabolic effects (66, 69). Sweetened drinks containing either sucrose or sucrose in combination with fructose appear to increase energy intake and lead to weight gain (72-74). There is some evidence that fructose might also be preferentially metabolized into fat (75). On the other hand, a review of literature demonstrates that fructose, as commonly consumed in mixed carbohydrate sources, does not have specific metabolic effects that can account for an increase in body weight and recommends that people should change their dietary behavior to reduce energy intake (76).

Sugar-sweetened beverages raise concern because they are energy - dense and yet produce low satiety (77). In fact, there is a strong correlation between the consumption of energy as a liquid and total energy intake. People do not tend to reduce energy intake in solid foods to compensate for increased energy intake in liquids (45). On the national scale, the American Heart Association estimates that about half of the total energy intake increase over the past 30 years is attributable to the energy content of liquids (69). The high glycemic load of these beverages is also thought to be a contributor to many chronic diseases, such as obesity (69, 76), diabetes (68), nonalcoholic fatty liver disease (78), the metabolic syndrome (69, 79, 80) and cardio metabolic problems (69) .

Between1977-1996, standard serving sizes have increased from 237ml to 355ml bottles (13). In the US, the percentage of total daily energy intake from energy containing beverages has almost doubled from 1965 to 2002 and in 2002 has reached to 21.0 %(81). Analysis of data from the PREMIER trial showed that a decrease of 1 serving per day of SSBs was associated with a 0.49kg decrease in weight at 6 month (82).

Replacing sugar-sweetened beverages that supply a substantial portion of daily energy with a lower energy alternative may be a strategy for modest weight reduction or weight-gain prevention, which would provide health benefits (83). Although diet beverages may be ideal targets (84, 85), replacement of diet beverages for energy containing beverages will be beneficial only if compensation, by consuming the energy from other foods, does not occur.

1.3.4 Sugar-sweetened beverages in children and adolescent

The increased prevalence of obesity in children and adolescents, which is a major health problem (86, 87) has occurred concurrently with a large increase in the consumption of sugar-sweetened beverages (81). These beverages are also considered to be more fattening than solid foods because they do not lead to a sense of satiety (88).

In recent years, the role of SSBs in promoting obesity has been debated widely. The American Academy of Paediatrics (89) and the current Dietary Guidelines for Americans support reducing SSB consumption as a weight-control strategy based on valid prospective data from cohort studies (90-92).

For comparative purposes, a prospective observational study found that BMI was raised by 0.24 kg/m^2 for every additional serving of SSBs consumed per day (90).

In a 1-year cohort study, Welsh et al (93) reported that preschool children who were overweight or at risk of overweight were ~2 times more likely to remain or become overweight, if they consumed SSBs. However, there was no significant association between SSBs consumption and weight gain in children who were not at risk for being overweight (BMI <85th percentile).

1.3.5 Water consumption and weight loss

Nutritionists and other weight management practitioners often advise people who wish to lose weight to increase their water consumption, and this strategy is often recommended in popular weight loss programs (94, 95). The general public recognize increasing daily water consumption as a weight loss strategy (96). There is, however, still little data supporting this practice.

Observational evidence suggests that drinking water is associated with weight loss and a reduction in energy intake (97-100). In the laboratory setting, outcomes of consuming a preload amount of water before meal ingestion has led to varying results by age, with no effect on meal consumption among younger adults (102) but less energy consumed by middle-aged and older adults (73, 102).

In terms of the effects of water consumption on energy intake (EI), as a factor of weight control, epidemiological studies suggest that EI is lower (~9%, or 194 kcal/d) in water drinkers when compared with non- water drinkers (103). However, interventional studies have failed to find conclusive evidence with respect to the effects of water consumption on EI and weight loss over time. An interventional study reported that replacing energy-containing beverages with water decreases self-reported EI (84), and that increasing self-reported daily water consumption by ≥ 1l/d in overweight women is associated with a greater weight loss of ~2 kg over a 12-month diet intervention compared with women who consumed < 1l/d of water (105). However, laboratory-based test meal studies have shown that whilst water consumed with a meal decreases ratings of hunger and increases rating of satiety (84, 106), no differences in meal energy intake were observed when compared with a no beverage condition (84). On the other hand, it was shown that both normal-weight and overweight/obese middle-aged and older adults ingest less energy at an ad libitum meal when given, 30 min prior to the meal, a water preload (500 ml) in comparison with a no-preload meal condition(94, 95). However, a reduction in meal EI following water consumption has not been observed in studies on young adults (107, 108), which suggests there may be age-related differences in the ability of water to reduce EI. Other studies also reported no effect of water consumption on EI in young adults (97, 109).

Dennis and colleagues tested the hypothesis that pre-meal water consumption would lead to greater weight loss in older overweight and obese individuals consuming a hypoenergetic diet. Their results showed that when combined with a hypoenergetic diet, consuming 500 ml (~16 fl oz) of water prior to each of the three main daily meals (1.5 l/d) leads to ~2 kg greater weight loss over 12 weeks when compared with a hypoenergetic diet alone, among middle-aged and older adults (96).

In a secondary analysis of a trial comparing several weight loss diets, Stookey *et al* (105) discovered that overweight women who reported drinking >1 l/d of water over a 12-month period lost~2 kg more than those who did not increase water consumption. However water consumption was not manipulated, and water consumption data was self-reported. Overall, data from Dennis *et al* are in agreement with these findings in that they support a beneficial role of increasing water consumption, while consuming a hypoenergetic diet.

Although the exact mechanism responsible for the greater weight loss with increased water consumption is unknown, consuming water before a meal or with a meal decreases sensations of hunger, and increases satiety (106, 110). Initially, changes in subjective sensations of hunger and satiety are associated with an acute reduction in meal EI (107, 110). Secondly, replacing energy-containing beverages in the diet with water may cause a reduction in overall EI, as epidemiological data suggests that total beverage energy contributes >400 kcal to daily EI in adults (81).

1.3.6 Artificial sweetener beverages in adults and body weight

Due to the negative effects of SSBs on weight control (67, 68), beverage industries have attempted to introduce diet beverages to allow customers to continue to drink their products. Considering the rise in the prevalence of obesity globally (111), it is important to establish whether artificial sweeteners can help control body weight. Reducing sugar sweetened beverages, with replacement by artificially sweetened beverages, offers an alternative strategy to introducing more water into the diet. People who are at risk of gaining weight may turn to artificially sweetened drinks in an attempt to decrease energy intake (112, 113). However consumers of artificially sweetened beverages may believe that the use of such sweeteners permits them to eat more of other foods which may cause a net rise in total energy intake (113). Today, there is still no agreement on the advantages associated with replacing sucrose with artificial sweeteners to obtain better weight control (114). Most of the published studies on artificial sweeteners are laboratory based or are short-term studies (73, 97-101, 108, 109, 115-117).

Short term intervention studies (not more than 3 week interventions) on the effect of artificial sweeteners in adults suggested that raised intake of artificial sweeteners either has no effect (102) or reduces energy intake (73) compared with sucrose consumption on their habitual diet, whilst the intervention time is not enough to reveal any significant change in weight. On the other hand, a few of these studies found a stimulating effect of artificial sweeteners on appetite (73, 115, 116). It has been suggested that removing sucrose from the diet will increase the relative dietary fat content (118, 119), which may then result in raised energy intake and body weight in the long term (120, 121).

Concerning artificial sweeteners, a long-term epidemiological study reported a positive relation between saccharin intake and weight change in adults (122). In contrast, long-term intervention studies with energy restriction have shown that the inclusion of artificial sweeteners can increase compliance, improve quality of life, and help maintain weight loss (123).

Raben at al investigated changes in ad libitum energy and macronutrient intakes, body weight, and body composition during 10 weeks of supplementation with either sucrose or artificial sweeteners (124). This study showed that energy intake, body weight, fat mass, and blood pressure rose after 10 weeks of supplementation with sucrose, whilst a decrease or no change in these variables was seen with supplementation with artificial sweeteners. These findings were not expected on the basis of previous intervention and observational studies (121, 122, 125, 126). Generally, female overweight subjects who were given supplemental drinks and foods containing sucrose, for 10 weeks, experienced rise in total energy intake, body weight, FM, and blood pressure. This was not observed in a similar group of subjects given similar drinks and foods containing artificial sweeteners. The most likely reason for these differences between the groups was the use of large amounts of beverages, giving rise to overconsumption of energy on the high-sucrose diet. Therefore, this study can support this comment that overweight individuals may want to consider choosing beverages containing artificial sweeteners rather than sucrose to prevent weight gain (124).

For the purposes of weight loss, replacing SSBs with DBs might promote greater adherence because of the availability of a variety of flavors and properties (carbonated, caffeinated, and sweetened) similar to the SSBs, which leads to greater adherence.

Debora and colleagues conducted a randomized controlled trial to use non-caloric beverage replacements, either water or diet beverage, alone as the primary weight-loss strategy in overweight adults and to examine two non-caloric alternatives (127). A significant reduction in weight and waist circumference and an improvement in systolic blood pressure were observed during 6 months in all groups without any significant differences between the groups. A significant reduction in fasting glucose at 6 month (*P* = 0.019) was observed in the water group relative to the control group. However, the authors concluded that in a combined analysis, participants assigned to beverage replacement were two times as likely to have achieved a 5% weight loss as were the control participants (127). They also suggested that replacement of caloric beverages with non-caloric beverages as a weight-loss strategy resulted in average weight losses of 2% to 2.5%. They proposed that on a population level, replacement of caloric beverages with non-caloric alternatives could be an important message for weight control. They also recommended that replacing SSBs with either DBs or water, based on the consumers' preference and ability to adhere, appears warranted to enable weight loss at this stage of research on the basis of these findings. However, these recommendations are not supported by the results of this study, since there is no significant difference in weight loss between replacing SSBs with water or DBs over the 6 month intervention (127).

Also, in a recently published study (128) was claimed that obese people drinking diet beverages lose significantly more weight compared with the water group while they were on a cognitive behavioral therapy (CBT) weight loss plan without any personalized detailed dietary recommendations for 12 weeks.

1.3.7 Artificial sweetener beverages in children and adolescents and body weight

Clarifying the role of sugar free and sugar containing beverages in children and young people may be particularly important because of their high and increasing consumption rates. Based on data from a nationally representative sample of youth, an extraordinary 73% of adolescent boys and 62% of adolescent girls consume carbonated soft drinks, of which the vast majority contain sugar rather than non-nutritive sweeteners, on any given day (129). Those who consume soft drinks obtain 10% to 11% of their total energy intake from these beverages (129). Soft drinks are the leading source of added sugars in the diets of adolescents (130).

Ebbeling et al (131) focused specifically on SSB consumption, as a single dietary behaviour which may have a particularly large impact on body weight in adolescents. The experimental group received home delivery of non-caloric beverages, and the control group did not. The mean body-mass index decreased significantly in the experimental group, as compared with the control group, only among the overweight and obese adolescents. Moreover, they used a novel environmental intervention, in combination with telephone-administered behavioural counselling, to penetrate homes and thereby achieve behaviour change. This study showed that reducing SSBs consumption had a beneficial effect on body weight that was strongly linked with baseline BMI. Net BMI change was -0.75 ± 0.34 kg/m^2 in the intervention compared with the control group among subjects in the upper baseline-BMI but BMI changes did not differ significantly between groups among subjects with lower baseline body weight. Furthermore, the effect was greater among the subjects who drank more SSBs at baseline, presumably because of greater displacement of SSBs by no caloric beverages. BMI decreased by −0.14 kg/m^2 for every 420 kJ (100 kcal) per day from SSBs at baseline. Because each 360-mL (12-fl oz) serving of SSBs contains ~630 kJ (150 kcal), and total SSBs consumption was decreased by 82% in the intervention group.

Cara et al (16) found that a supply of non-caloric beverages almost eliminated reported consumption of sugar-sweetened beverages and decreased total reported energy intake between overweight and obese adolescents after a 1-year intervention, and there were constant effects on diet through follow-up at 2 years. The change in BMI differed significantly between the experimental and control groups at 1 year but not at 2 years.

De Ruyter et al (132) found that hidden replacement of a sugar-containing beverage with a sugar-free beverage markedly decreased weight gain and body fat gain in healthy children.

On the other hand, a recent review raised a doubt about the long term positive effects of diet beverages on weight control in children (112, 113).

It seems that whatever is the explanation, the epidemiological association of the use of artificial sweeteners with obesity reveals that switching to artificially sweetened beverages by itself is insufficient to overcome weight gain.

1.4 Obesity management

More than two-thirds of adults in the United States are either trying to lose weight or to maintain their weight (133). The optimal management of overweight and obesity needs a combination of diet, exercise, and behavioural modification.

Treatments for obesity either reduce energy intake or increase energy expenditure. Those that decrease energy intake have a greater potential for triggering weight loss than those that increase energy expenditure through exercise.

Behaviour modification or behaviour therapy is one keystone in the treatment for obesity. These concepts are usually included in programs conducted by psychologists or other trained personnel as well as many self-help groups.

Generally, all patients who are overweight (BMI ≥25 kg/m^2) or obese (BMI ≥30 kg/m^2) should take counselling on diet, lifestyle, and goals for weight management. The choice of therapy is dependent on several factors including the degree of overweight or obesity and patient preference.

1.4.1 Setting goal for weight loss

It is very important to set goals when discussing a dietary weight loss program with an individual patient. An initial weight loss goal of 5 to 7 percent of body weight is realistic for most individuals (134). It has been shown that a weight loss of more than 5 percent can reduce risk factors for cardiovascular disease, such as dyslipidaemia, hypertension, and diabetes mellitus (135).

The rate of weight loss is directly related to the difference between the subject's energy intake and energy requirements. Decreasing energy intake below expenditure results in a predictable initial rate of weight loss that is related to the energy deficit (136).

Approximately 22 to 25 kcal/kg is required to maintain body weight in a normal adult (137). Therefore, the expected or calculated energy expenditure for a woman weighing 100 kg is about 2200 to 2500 kcal/day. The variability of ±20 percent could give energy needs as high as 2620 to 3000 kcal/day and as low as 1860 to 2000 kcal/day. An average deficit of 500 kcal/day should result in an initial weight loss of approximately 0.5 kg/week (1 lb. /week). However, after 3 to 6 months of weight loss, energy expenditure adaptations happen, which slow the bodyweight response to a given change in energy intake, thereby reducing ongoing weight loss unless energy intake is further reduced (138).

1.4.2 Types of diets

The general agreement is that excess intake of energy from any source, associated with a sedentary lifestyle, causes weight gain and obesity. The goal of dietary therapy, therefore, is to decrease energy intake from food. Conventional diets are defined as those below energy requirements but above 800 kcal/day (139). These diets fall into four groups:

- Balanced low-calorie diets/portion-controlled diets
- Low-fat diets
- Low-carbohydrate diets
- Mediterranean diet

1.4.2.1 Balanced low-calorie diets/portion-controlled diets

Diet planning requires the selection of a target energy intake and then selection of foods to meet this intake. It is essential that foods are consumed that will meet all nutritional requirements (except for energy), including protein, carbohydrate and essential fatty acids, as food restriction will in many instances be a long term necessity. Thus, weight-reducing diets should eliminate alcohol, sugar-containing beverages and most highly concentrated sweets because they rarely contain adequate amounts of other nutrients besides energy.

Breakdown of some protein is to be expected during weight loss. When weight increases as a result of overeating, approximately 75 percent of the extra energy is stored as fat and the remaining 25 percent as lean tissue (97). If the lean tissue contains 20 percent protein, then 5 percent of the extra weight gain would be protein. Thus, it should be anticipated that during weight loss, at least 5 percent of weight loss will be protein. A desirable feature of any energy-restricted diet, however, is that it results in the lowest possible loss of protein, recognizing that this will not be less than 5 percent of the weight that is lost.

One simple approach to providing a calorie-controlled diet is to use individually packaged foods, such as formula diet drinks using powdered or liquid formula diets, nutrition bars, frozen food, and pre-packaged meals that can be stored at room temperature as the main source of nutrients.

Frozen low-calorie meals containing 250 to 350 kcal/package can be a suitable and nutritious way to do this. Often, it has been recommended the use of formula diets or breakfast bars for breakfast, formula diets or a frozen lunch entree for lunch, and a frozen calorie-controlled entree with additional vegetables for dinner. In this way, it is possible to obtain a calorie-controlled 1000 to 1500 kcal per day diet. In one four-year study this approach resulted in early initial weight loss, which then was maintained (140).

1.4.2.2 Low-fat diets

Low-fat diets are another standard plan to help patients lose weight, and almost all dietary guidelines recommend a reduction in the daily intake of fat to 30 percent of energy intake or less (141).

A low-fat diet can be applied in two ways. First, the dietician can provide the patients with specific menu plans that highlight the use of reduced fat foods. Second, patients can be instructed in counting fat grams as an alternative to counting calories. Fat has 9.4 kcal/g. It is thus very easy to calculate the number of grams of fat a subject can eat for any given level of energy intake but not as easy to know how much food this represents.

Many experts recommend keeping energy from fat to below 30 percent of total calories. In practical terms, this means eating about 33 g of fat for each 1000 kcal in the diet.

1.4.2.3 Low-carbohydrate diets

The carbohydrate content of the diet is an important determinant of short-term (less than two weeks) weight loss. Low (60 to 130 grams of carbohydrates) and very low-carbohydrate diets (0 to <60 grams) have been popular for many years (139).

Low and very low-carbohydrate diets are more effective for short-term weight loss than low-fat diets, although perhaps not for long-term weight loss (142). Low-carbohydrate diets may have some other beneficial effects with regard to risk of developing Type 2 diabetes mellitus, coronary heart disease, and some cancers, particularly if attention is paid to the type as well as the quantity of carbohydrate. A low-carbohydrate diet can be implemented in two ways, either by reducing the total amount of carbohydrate or by consuming foods with a lower glycaemic index or glycaemic load.

1.4.2.4 Mediterranean diet

The term Mediterranean diet refers to a dietary pattern that is common in olive-growing areas of the Mediterranean area. Although there is some variation in Mediterranean diets, there are some common components that include a high level of monounsaturated fat relative to saturated; moderate consumption of alcohol, mainly as wine; a high consumption of vegetables, fruits, legumes, and grains; a moderate consumption of milk and dairy products, mostly in the form of cheese; and a relatively low intake of meat and meat products.

A meta-analysis of six randomized controlled trials discovered no difference between three of the main diet types (low calorie, low carbohydrate, and low fat), with a 2–4 kilogram (4.4–8.8 lb.) weight loss in all studies (143).

1.4.3 Weight maintenance diets

Although many individuals have success losing weight with diet, most subsequently regain much or all of the lost weight. Maintaining weight loss is made difficult by the reduction in energy expenditure that is induced by weight loss (144). In addition, long-term adherence to restrictive diets is difficult. Exercise and behavioural interventions may help individuals maintain weight loss.

There is little agreement on the ideal mix of macronutrients to maintain weight loss. The satiating effects of high protein, low glycaemic index diets have generated interest in manipulating protein composition and glycaemic index in weight maintenance diets.

Whether the macronutrient composition of a diet, independent of total energy intake, has any effect on long-term weight maintenance is unknown. As discussed above, long-term adherence to a weight maintaining diet is probably the most important determinant of success, and therefore the optimal weight maintaining diet will depend upon preference and individual factors.

1.4.4 Obesity and physical activity

Physical activity is a component of energy balance that is particularly important in the pathogenesis of obesity and in its treatment. Activity-related energy expenditure can be defined as any situation involving muscle contraction which increases energy expenditure above the resting supine or seated level.

The best management of overweight and obesity starts with a combination of diet, exercise, and behavioural modification. Physical exercise and activity can be beneficial in preserving lean body mass while dieting.

Exercise has been assessed as a single treatment for obesity, in combination with diet, and for maintaining weight loss after successful achievement of a weight loss program. In most studies, exercise only marginally improves weight loss. The reasons for this finding are unclear but may be due, partly to difficulty in adhering to adequately vigorous exercise schedules. In addition, for patients who are adherent with exercise training, increases in muscle mass may offset loss of adipose tissue, resulting in less net weight loss.

Exercise programs added to diets with moderate to severe energy restriction have little additional effect upon weight loss. As an example, a systematic review of 17 randomized trials in overweight and obese patients (BMI 25 to 37 kg/m^2) showed that compared with weight loss by diet alone, adding exercise to diet achieved slightly greater weight loss than diet alone (mean increase in weight loss approximately 1.5 kg)(145). However the results were statistically significant in only two of the trials. Similar findings were later reported in obese elderly adults (mean age 70 years) who were randomly assigned to diet or exercise alone, diet plus exercise, or control (146). Body weight decreased equally in the diet alone and the diet plus exercise group (10 and 9 percent, respectively), but did not decrease in the exercise alone or control groups.

Also, in observational studies, exercise regularly stands out as an important factor in maintaining weight loss after any weight reduction (147-150). The amount of physical activity which is required to prevent weight regains seems to be >150min/week (151).

1.5 Type 2 diabetes and its management

Type 2 diabetes is a mixed group of conditions that represent about 90% of all diabetes cases in the United States (152). Previously, Type 2 diabetes was known as non–insulin-dependent diabetes or adult-onset diabetes. Type 2 diabetes mellitus is characterised by hyperglycaemia, insulin resistance, and relative impairment in insulin secretion, compared to the absolute insulin deficiency seen in Type 1 diabetes.

Type 2 diabetes is a common disorder with a prevalence that rises significantly with increasing degrees of obesity (153). The prevalence of type 2 diabetes has risen alarmingly in the past decade (154). The estimated overall prevalence of diabetes among adults in the United States ranges from 5.8 to 12.9 percent (155). In large part linked to the trends in obesity and sedentary lifestyle (156).

Diabetes accounts for nearly 14 percent of United States health care expenditures, at least one-half of which are linked to complications such as myocardial infarction, stroke, end-stage renal disease, retinopathy, and foot ulcers (157). Numerous factors, in addition to directly related medical complications, contribute to the impact of diabetes on quality of life and economics.

Diabetes is associated with a high prevalence of depression (158) and adversely impacts employment, absence, and work productivity (159).

The management approach is consistent with guidelines from the American Diabetes Association (ADA) for health maintenance in patients with diabetes, which are published yearly (170). The majority of patients with this form of diabetes are clinically obese, and exercise and weight loss lead to improvements in the disease state.

The first Survey of Risk Factors of Non-Communicable Diseases of Iran, 2005, showed that 7.7% of adults aged 25–64 years, or 2 million adults, have diabetes, among whom one-half are undiagnosed. An additional 16.8%, or 4.4 million, of Iranian adults have impaired fasting glucose (160).

Haghdoost’s review indicated that the prevalence of Type 2 diabetes in those > 40 years was 24% and it increased by 0.4% with each year after 20 years of age. The risk of Type 2 diabetes was 1.7% greater in women than men. The prevalence of Type 2 diabetes seems higher in Iran than in other developing countries but because of differences in age pyramids, the rough prevalence is not a proper indicator and age-adjusted or age-specific prevalence should be used (161).

1.5.1 Causes of Type 2 Diabetes

Patients present with a combination of varying degrees of insulin resistance and relative insulin deficiency, and it is likely that both contribute to type 2 diabetes (162-164). Each of the clinical features can arise through genetic or environmental effects, making it difficult to control the exact cause in an individual patient. Besides, hyperglycemia itself can impair pancreatic beta cell function and exacerbate insulin resistance, leading to a vicious cycle of hyperglycemia causing a worsening metabolic state (165).

Generally, type 2 diabetes has several causes: genetics and lifestyle are the most important ones. A combination of these factors can cause insulin resistance, when the human body does not respond to insulin as well as it should. Insulin resistance is the most common cause of type 2 diabetes. Insulin resistance is assumed to precede insulin deficiency in most patients, and autoimmune destruction of β-cells does not happen, although β-cell mass may be reduced (166).

Genes play an essential role in type 2 diabetes, but lifestyle choices are also important. Someone can, for example, have a genetic mutation that may make him/her susceptible to type 2 diabetes, but if this person takes good care of his/her body, development of diabetes may be delayed or even prevented.

Type 2 diabetes is often accompanied by other conditions, including hypertension, high serum low-density-lipoprotein (LDL) cholesterol concentrations, and low serum high-density-lipoprotein (HDL) cholesterol concentrations that, like Type 2 diabetes, increase cardiovascular risk. This cluster of clinical conditions is referred to as the metabolic syndrome (167).

1.5.2 Screening test for Type 2 diabetes

The most frequently used screening tests for type 2 diabetes include measurement of fasting plasma glucose (FPG), two-hour post prandial plasma glucose during an oral glucose tolerance test (2-h OGTT), and glycated haemoglobin (HbA1C) .

The sensitivity and specificity of FPG and HbA_{1C} as screening tests differ according to the population tested and the threshold used to explain diabetes. Using a two-hour blood glucose >200 mg/dl (11.1 mmol/l) on an oral glucose tolerance test as the reference standard, the specificity of a fasting blood glucose ≥126 mg/dl (7.0 mmol /l) was greater than 95 percent; the sensitivity was about 50 percent, and may be lower for people over the age of 65 (168) . Using the same reference standard, the specificity and sensitivity of an HbA_{1C} cut point of 6.5 percent were 79 and 44 percent, respectively (169).

1.5.3 Lifestyle management of Diabetes

Lifestyle modifications and changes in day-to-day habits, are essential factors of any diabetes management plan. Lifestyle modifications can be a very effective method to keep diabetes in control. Improved blood glucose control can slow the progression of long-term complications. Multiple small changes can lead to improvements in diabetes control, including a reduced need for medication (170).

Diabetes requires a lifelong management plan, and persons with diabetes have a central role in this plan. Lifestyle modifications are an opportunity for diabetics to take charge of their health. Therefore, it is important to learn as much as possible about diabetes and to take an active role in making decisions about healthcare and treatment.

Diet, weight reduction, and exercise can all be used to improve glycaemic control. In the Diabetes Prevention Program, a multi-centre trial in patients with impaired glucose tolerance, weight loss of 7 percent reduced the rate of progression from impaired glucose tolerance to diabetes by 58 percent (171).

1.5.3.1 Exercise and Type 2 diabetes

Exercise is beneficial for all people, with or without diabetes. Even people with longstanding diabetes or diabetic complications can benefit from exercise. For diabetics, exercise promotes cardiovascular fitness and weight loss, lowers high blood pressure, improves lipid profiles, improves blood glucose control in some cases, and leads to a general sense of well-being.

The American Diabetes Association (ADA) recommends (170) that patients who plan to begin a moderate- to high-intensity exercise program undergo screening for cardiovascular disease if they are > 35 years of age or if they are > 25 years of age and have had type 2 diabetes for > 10 years or type 1 diabetes for > 15 years, have an additional risk factor for coronary disease, or have microvascular disease, peripheral vascular disease, or autonomic neuropathy. Decisions regarding screening of patients who plan low levels of physical activity, such as walking, are left to the discretion of their treating physicians (172).

Type 2 diabetes patients should understand that the amount of exercise that produces a beneficial effect on health is not large; as little as 30 minutes of moderate physical activity daily may offer protection from diabetes (173). In patients with Type 2 diabetes, structured regimens of physical activity for 8 weeks or longer improved HbA_{1C} independent of changes in body mass. There may also be further improvement in HbA_{1C} with increasing intensity of exercise (174, 175).

1.5.3.2 Macronutrient Considerations in Type 2 diabetes

A. Dietary Carbohydrate

It is recommended that about 60% of total energy intake in people with type 2 diabetes should come from carbohydrate or monounsaturated fat. However, there is some concern that increased unsaturated fat consumption may promote weight gain in obese patients with type 2 diabetes and so reduce insulin sensitivity (176). Glycemic responses appear to be similar between starches and sucrose and, therefore, sucrose does not need to be eliminated from the diet (177).

The "glycemic index" concept compares the glycemic effects of various foods to a standard, such as white bread. While several authors have proposed its clinical efficacy in controlling postprandial hyperglycemia, several prospective studies have not confirmed a clear improvement in HbA_{1C} in patients using low–glycemic index diets (176). Nevertheless, the recent meta-analysis studies on the effects of low–glycemic index diets suggested a mild but significant improvement in HbA_{1C} (178, 179). Therefore, there may be a small benefit in following a low–glycemic index diet for patients with diabetes. This benefit, however, appears to be less than the benefit that can result from counting or controlling the total amount of carbohydrate consumed at meals.

Many sweeteners are available to the general public. The most common sweetener is sucrose. Studies comparing the effects of the same amounts of sucrose and starch on glycemic control have almost no differences (180). Sucrose should be adequately covered by short-acting insulin at mealtime but does not need to be eliminated from the diet (181). Fructose may cause less postprandial hyperglycemia, but there is some evidence suggesting that it may also lead to or worsen hyperlipidemia (182). Therefore, adding fructose to the diet as a sweetening agent is not recommended by the ADA, although naturally occurring fructose in foods such as fruits does not need to be avoided (176, 179).

The U.S. Food and Drug Administration (FDA) has approved several sugar alcohols for use as sweeteners. These include products such as sorbitol, a common sweetener in chewing gum. Sugar alcohols cause less hyperglycemia than naturally occurring sugars and may also reduce the risk of dental caries. They are only partly absorbed from the intestinal tract and so may lead to diarrhea or gastrointestinal discomfort, especially if consumed in higher amounts (183). They provide almost half the energy of natural sugars (due to colonic fermentation) and should be included in carbohydrate counting at half the effect of sucrose. These products have not been shown to facilitate weight loss or improve glycemic control (176, 179). Several sweeteners that are nonnutritive and do not affect blood glucose levels are also available to the public (which have been mentioned in 1.3.2). These include aspartame, sucralose, saccharin, neotame, and acesulfame potassium. Though formerly linked to carcinogenesis in laboratory animals at very high doses, saccharin is no longer considered a cancer-causing chemical by the FDA (176). One of the most recently released sweeteners, sucralose, has been revealed to have no significant effect on blood glucose levels and so may be omitted from carbohydrate calculations (176, 179, 184). Use of these sweeteners has not been shown to facilitate weight loss or improve glycemic control in type 2 diabetes.

B. Dietary Protein

Although the majority of clinical attention in the management of patients with diabetes is on carbohydrate metabolism, protein metabolism in the state of diabetes is also abnormal. Patients with type 2 diabetes exhibit a more negative nitrogen balance than normal subjects. Protein poverty appears to be worsened by hyperglycemia and improved by controlling glucose levels with insulin therapy (185, 186).

Protein requirements for people with type 2 diabetes may be slightly larger than those for non-diabetic persons. However, most individuals in the United States consume considerably more protein that the recommended daily allowance (176). Studies of patients with type 2 diabetes have confirmed that protein consumption does not increase plasma glucose concentrations and that endogenous insulin release is stimulated by protein consumption (187). It is important to note that the existing studies of low-carbohydrate diets are short-term studies; the long-term effects of such diets are unknown. This is especially concerning because of their wide-spread use and the association of diabetic kidney disease with diets consisting of > 20% of energy from protein. For these reasons, a low-carbohydrate diet (< 130 g of total carbohydrate per day) is not recommended by the ADA (179).

C. Dietary fat

Recommendations regarding fat in the diet of type 2 diabetes are similar to recommendations for patients with coronary artery disease. This is mainly because of studies that show that the risk of myocardial infarction in diabetic patients is similar to the risk of non-diabetic patients who have previously suffered a myocardial infarction (188). As saturated fats are the major dietary factors of serum LDL cholesterol levels, people with diabetes should struggle to keep them to < 7% of total daily energy and to minimize consumption of trans fatty acids. Cholesterol consumption should be < 200 mg/day (179).

When combined into a controlled-calorie diet in which no weight loss is occurring, programs that highlight reduction in either carbohydrate or monounsaturated fats both lower cholesterol, but the higher-carbohydrate diets may worsen hyperglycemia. In diets in which total energy was reduced to help weight loss, however, the hyperglycemic effect of the high-carbohydrate diet seemed lessened (179).

1.6 Summary, proposed hypothesis and topic of thesis

Total energy consumption has increased in recent decades (189), and sugar sweetened beverages (SSBs) potentially contribute to this increase (190). Raised consumption of SSBs is frequently associated with increased cardiometabolic disorders, specifically with obesity and metabolic syndrome around the world (69). Some health professionals recommend that a decrease in consumption of SSBs may have a positive effect on weight control (191), but this has not been confirmed by others (76). Low-energy sweeteners are of interest as a dietary tool (192), as they offer sweet taste without the extra energy delivered from foods and drinks containing sugar (193-198) and promote weight-loss program adherence (199). However, some studies presented an opposing outcome of such sweeteners on energy intake and body weight (200) and another study

has suggested that regular use of intense sweeteners might lead to a paradoxical weight gain (201).

On the other hand, increasing daily water drinking is generally recognized as a weight loss strategy by the general public but there are few data available to support this practice. There is a belief among nutritionists and health professionals that increasing daily water consumption is a way to help weight loss, but there are few data available to support this practice. Epidemiological studies suggest that energy intake is significantly lower in water drinkers compared with non-water drinkers (201) which may contribute to weight gain. Previous interventional studies suggest water consumption in subjects following a weight loss diet may lead to more weight loss compared with the control (96, 202). On the other hand, a recent study indicated that replacement of SSBs with non-caloric alternatives, i.e. either DBs or water, may have similar effects on weight loss (127).

The general population believes that diet beverages can help them to lose weight and many obese people drink diet beverages, believing that this simple strategy would be helpful to reduce weight. The study of Tate and colleagues (127) has claimed that replacement of energy containing beverages with diet beverages resulted in average weight losses of 2% to 2.5% without any weight loss diet program. Nonetheless, this study failed to indicate any significant differences in weight loss during 3 and 6 month interventions when replacing diet beverage or water for SSBs compared with the control group. However, fasting blood glucose was only significantly reduced in the water group, not the diet beverage group, compared with control. Furthermore, a recent study (128), reported concurrently with the studies presented in this thesis, claimed that obese people drinking DBs even lost significantly more weight than the water group while they were on a cognitive behavioral therapy (CBT) weight loss plan for 12 wk., without any personalized detailed dietary recommendations.

The 1 year follow-up of the study also confirmed that regular consumers of NNS beverages who were asked to drink NNS beverages lost significantly more weight, and maintained significantly greater weight losses, over 1 year than subjects stopping NNS beverage consumption and drinking water only (203).

To my knowledge, no study compared the effects of replacing diet beverages with water on weight loss management in people who usually drank diet beverage, following a comprehensive weight loss diet program for 24 weeks and in the long term. In summary, the purpose of this study was to examine the effects of replacing diet beverage consumption with water on different aspects of human energy metabolism in the three studies as below:

1. Effects on weight loss in adults of replacing diet beverages with water during a hypoenergetic diet-a randomized, 24 week clinical trial

2. Effects of replacing diet beverages with water on weight loss and weight maintenance: 18 month follow up, randomized clinical trial

3. Beneficial effects of replacing diet beverages with water on Type 2 diabetic obese women following a hypo-energetic diet - a randomized, 24 week clinical trial

Chapter 2.General Methods

2.1 Introduction

The studies included in this thesis have the following protocols, methods and measurement in common. These will be discussed in this chapter. Specific method and protocols will be explained in the subsequent appropriate chapters.

2.2 Participants and ethical approvals

The studies took placed in the NovinDiet clinic, Tehran, Iran between March 2014 and December 2015. All the studies were approved by the Ethical Committee of The Digestive Research Institute, Tehran University of Medical Science. All participants gave informed written consent to participate in each study.

2.2.1 Participants recruitment

Female participants were selected from overweight and obese patient attending NovinDiet Clinic to lose weight. The following criteria for selection were used:

- Age=18-50 years of age
- BMI=27-40kg/m² in healthy and BMI=27-35 kg/m² in the diabetic subjects study
- Neither pregnant nor lactating during the previous 6 months or planned pregnancy in the next 6 months
- No weight loss ≥10% of body weight within the 6 months before enrollment in the study
- No participation in a research project involving weight loss or physical activity in the previous 6 months
- Not taking medication to lower lipids/cholesterol
- Nonsmokers

- Free of established cardiovascular diseases, stroke, liver diseases, kidney diseases, cancer or autoimmune disease.
- Self-reported habitual consumers of DBs (diet coke) who were willing to introduce a dietary change to lose weight which might include changing beverage consumption.
- No indication of current depression: identified by a score of less than 10 on the Beck depression Inventory (BDI), (see appendix-5).The questionnaire was developed to measure the symptoms of depression using 21 items with four levels of severity associated with each symptom (204).
- Participants reporting heart disorders, frequent chest pains, or faintness or dizziness on the Physical Activity Readiness Questionnaire were excluded, and other medical conditions required physician's consent to participate.

2.2.2 Protocol for screening visit

The screening visit took up to 100 minutes for each participant. The history of the patient was collected (section 2.2.2.1). Also weight, height and waist circumference were measured (section 2.2.2.2) and. Subjects were asked to complete a questionnaire (2.2.2.3) and asked them to fill a food dairy diary form during the next week for 3 weekdays and one weekend day (2.2.2.4).

2.2.2.1 Collecting patient’s history

Participants underwent a physical examination by a general medical doctor. The practitioner asked questions to obtain the following information about the patient:

- The "chief complaint (CC)" - the major health problem or concern, and its time course.
- History of the present illness (HPI) - details about the complaints, counted in the CC. (Also often called 'History of presenting complaint' or HPC)
- The medical history of participants and their family. If they are taking any medication.
- Past medical history (PMH) (including major illnesses, any previous surgery/operations (sometimes distinguished as "Past Surgical History" or PSH), any current ongoing illness, e.g. diabetes).
- Family diseases - especially those relevant to metabolic syndrome.
- Childhood obesity.
- Social history (medicine) - including living arrangements, occupation, marital status, number of children, drug use (including tobacco, alcohol, other recreational drug use).
- Regular and acute medications (including those prescribed by doctors, and others obtained over-the-counter or alternative medicine).
- Allergies - to medications, food, latex, and other environmental factors
- Sexual history, obstetric/gynacological history, and so on, as appropriate.

(See appendix-1)

2.2.2.2 Anthropometric measurements

I. Body weight was taken to the nearest 0.1 kg using a digital calibrated scale (Omron Health Care, Hoofddorp, Netherland), whilst subjects wore light clothing and no shoes.

II. Body height was measured to the nearest 0.1 cm by using a wall mounted stadiometer (SECA, Hamburg, Germany) while participants were barefoot and in a free-standing position.

III. Waist circumference (WC) was measured with a rigid measuring tape and recorded to the nearest 0.5 cm. WC was measured at the smallest horizontal circumference between the ribs and iliac crest (the natural waist), or, in case of an indeterminable waist narrowing, halfway between the lower rib and the iliac crest (205).

IV. BMI was calculated from measured weight in kilogram divided by the square of height in meter.

2.2.2.3 Questionnaires

Participant was asked to complete the following questionnaire at the screening visit:

I. A food frequency questionnaire (FFQ) which is a limited checklist of beverages with a frequency response section for participants to report how often each item was consumed over a specified period of time (See Appendix-6).

II. Beck's Depression Inventory (BD1) (204) (See Appendix-5).

III. Physical Activity Readiness Questionnaire (PAR-Q) (206) (Appendix-4).

IV. Lifestyle and Eating Habits Questionnaire (See Appendix-3).

2.2.2.4 Completion of the food diary form

Participants were instructed in how to keep a 4- day food record by a research nutritionist. In the 30-minute lesson emphasis was placed on the necessity of time recording and accurate food and drink record keeping for 4 consecutive days which included 3 weekdays and 1 weekend day.

2.2.3 Protocol for Blood sampling

After the screening visit, anyone who was eligible to participate in the study was asked to come to the clinic for blood sampling. The blood samples were collected for all participants at week 0, and then at week 12 and/or week 24 according to the protocol of the experiments.

Participants were asked to fast overnight. Blood samples were taken while the participants were in a sitting position from an antecubital vein, and were centrifuged at 2000g at room temperature within 30–45 min. Antecubital venous blood samples for 2hpp glucose were taken 2 hours after ingesting 75g of glucose according to the standard method and the American Diabetes Association's criteria were used for excluding diabetes (207).

2.2.3.1 Blood glucose measurement

Fasting plasma glucose (FPG) and 2hpp plasma glucose levels were measured using the enzymatic colorimetric method.

Insulin was measured by using a radioimmunoassay with ^{125}I-labeled human insulin and a human insulin antiserum in an immunoradiometric assay (IRMA) (Biosource, Dorest, Belgium) with a gamma-counter system (Gamma I; Genesys). Insulin resistance was evaluated by homeostasis model assessment of insulin resistance (HOMA-IR), which was calculated by using the following formula:

HOMA-IR = [fasting insulin (mU/l) × FPG (mmol/l)]/22.5 (208)

Glycated hamoglobin (HbA_{1c}) was measured by a colorimetric method after an initial separation by ion exchange chromatography (Biosystem, Barcelona, Spain).

2.2.3.2 Lipid profile measurement

Biochemical analysis of the serum total cholesterol (TC), triglyceride (TG), and high-density lipoprotein (HDL) cholesterol was carried out on a Selectra E auto analyzer (Vita Laboratory, Netherlands) following standard procedures of the Pars Azmoon diagnostic kits (Iran). The LDL cholesterol was calculated using the Friedewald formula.

LDL cholesterol = TC − HDL cholesterol + (TG ÷ 2.2) (209)

2.2.4 Questionnaires

2.2.4.1 Beck's Depression Inventory (BDI) evaluation

By evaluating the Beck's depression inventory which the participant completed at the screening visit, participants were excluded if the scores were greater than 9.

2.2.4.2 Food diary record

The participants were asked to complete the food diary record at week 0, and then the last week of the interventions. The food diary records which were given at the screening visit and completed by the participant were evaluated for completeness at the next visit and subjects were excluded if it was not sufficiently detailed.

2.2.4.3 Lifestyle and Eating Habits Questionnaire

The participants were requested to complete the Lifestyle and Eating Habits Questionnaire at screening visit. This questionnaire was completed by the participant and then used by our dietitian to give each participant individual comments regarding necessary behavioral changes in their eating habits and lifestyle.

2.2.5 Protocol for clinic visit

2.2.5.1 Dietary and activity intervention sessions

NovinDiet Clinic is a private weight loss clinic which uses an integrated approach (dietary, behavioural, exercise and medical treatments). The clinic staff engage in research as well as providing the clinical services. Subjects who participated in studies did not pay clinic fees.

The NovinDiet Protocol is based around developing a problem solving approach for each member individually and addresses both diet and exercise. The program was designed to enable weight loss of 7-10% of starting body weight, at a rate of 0.5-1 kg/week over 24 weeks.

The individual diet programs were based on the participant's food diary records and their food preferences with gradual modification to bring their diet in line with the NovinDiet protocol. Participants were assigned a hypoenergetic diet with a mainly high-carbohydrate, low-saturated-fat dietary pattern [17% of energy from protein, 23% from fat (<10% from saturated fat), and 60% from carbohydrate.

Physical activity was encouraged; the objective was to gradually increase activity levels to achieve 60 minutes of moderate activity on five days each week. Predominant behavior change strategies applied included stages of change, goal setting, self-monitoring with food diaries, waist measurements, and physical activity.

At bi-weekly sessions, the participants reported behavior problems regarding their weight loss program were discussed. Resources were provided as home booklets for each person to record adherence to the diet protocol. During the intervention period, participants completed the feedback form regarding their adherence to diet and their physical activity (See Appendix-7) and also had access to a website, weekly internet magazines, and one to one telephone/ online support from a consultant, if needed.

2.2.5.2 Measurements

Anthropometric measurements were taken at the baseline, 12 weeks and 24 weeks for study1 and study2 and for study3 study at week 77(except height which was taken only at the screening visit), by the dietitian.

Blood samples were taken after overnight (10- 12 h) fasting by nurse, between 07:00 and 09:00, at the baseline, 12and 24 weeks for biochemical, cellular and hormonal measurements.

2.2.6 Free living protocol-food intake measurements

There are a number of different approaches to estimate energy intake (210). In my studies, a food dietary record based on household measurement method was used. The participants were given training in keeping a food diary record using this approach (See appendix-2). An instruction booklet with a day menu example was also given to each participant. During the 30-minute lesson, research nutritionists gave all necessary instruction to each person.

Methods used for food intake measurement are associated with errors rising from the use of food composition table (211), portion size estimation, daily diet variation and change in diet within the measured period (212). However it is claimed that a dietary record over 7 days is likely to reflect someone's habitual diet (213). In general, a record of three days is recommended to obtain valid information on food consumption. Further studies showed that energy intake was significantly different between weekdays and weekend days (214, 215). Therefore, to cover the potential errors caused by daily intake variations on energy intake estimation, the participants recorded their habitual dietary intake on 1 weekend day and 3 weekdays before the start of each study. Participants were asked to provide a food diary record for 4 days at Week 0, Week11 and Week 23 of the DBs and DDBs study and also at week 77 for DBs follow up study in order to measure their adherence to the diet during each interventional period. Food diary records for the Energy and macronutrient intake at the baseline, 11 weeks and 23 weeks (and 77 weeks) of the intervention was analyzed by Nutritionist IV software. Participants were asked to write exactly what they ate immediately at the end of eating any food or snack in order to make the records more accurate. They were also asked to keep the labels of any ready prepared food, which they consumed, and record the amount of which was eaten. The typical portion included in the software was used when portion size was not indicated on the record.

One of the main criticisms for the previous studies is the lack of the definition of key variables such as meals or snacks. For this thesis, a meal was defined as any food or snack (solid or liquid) containing energy, with an interval between two eating occasions of more than 1 hour. For the purposes of this thesis, it was decided that consumption of any energy containing food or drink would be classified as an eating occasion, either in a meal or snack.

Clearly, this random definition could lead to problems if participants consumed energy containing food/drink at 30 min. According to the prescribed diet, it was thought that it was unlikely that participants would be instructed to have their eating occasion with at least 1 hour intervals.

Participants were asked to note down the times at which the eating occasion occurred. It is appreciated that these definitions may lead to some debate, but they at least give opportunity to check any effects observed in other experiments with similar a protocol.

2.2.7 Statistical analysis

The data were processed using Excel software, version 2010. Following the study, results of anthropometrics measurement were processed in the computer. At the end of each study, results of food intake record, serum and plasma biochemical variables were processed.

All statistical analyses were performed using SPSS 22.0 for Windows (SPSS Inc., USA). All data are reported as mean ± their standard deviation (SD) unless otherwise indicated.

Data were tested for normality(Kolmogorov-smisnov).Baseline values of cardiovascular risk factors (including weight, waist circumference, LDL-c, HDL-c, TC, FPG, TG , fasting insulin, HOMA IR, HbA1C, 2hpp glucose and food intake data) were compared within the groups using unpaired *t*-tests.

At baseline, distribution was normal for all variables. Repeated-measures two-way analysis of variance (ANOVA) with groups and times as a within-subject factor was used to assess the effects of dietary intervention by comparing changes in the dependent variables between the groups over time. Where there was a significant main effect but no interaction, post-hoc comparisons were performed as appropriate with Bonferroni’s adjustment for multiple comparisons to determine differences between group means.

Statistical significance was set at $p \leq 0.05$. All data are presented as mean ± SD unless otherwise stated. Associations between variables were assessed by simple correlational analyses (Pearson's *r*).

Chapter 3. Effects on weight loss in adults of replacing diet beverages with water during a hypoenergetic diet-a randomized, 24 week clinical trial

3.1 Introduction

Low-energy sweeteners are of interest as a dietary tool by way of offering sweet taste without the extra energy brought from foods and drinks containing sugar, as an attempt to control obesity (192-198). Though, some studies showed an opposite result of Low-energy sweeteners on energy intake and body weight (200).

On the other hand, generally increasing drinking water is recognized as a weight loss plan by the general public however limited data are available to support this practice. To my knowledge, no previous study has compared the effects of replacing diet beverages with water on weight loss management in obese and overweight adult females, who usually drank diet beverages, following a comprehensive weight loss diet program for 24 weeks. The purpose of this study was to examine the effects of replacing diet beverage consumption with water on human energy metabolism in overweight/ obese habitual diet beverage consumers whilst undertaking a comprehensive 24 week weight loss program.

3.2 Methods

3.2.1 Subjects

Eighty nine healthy overweight and obese female participants were selected between March 2014 and June 2014 from participants attending NovinDiet Clinic, Tehran, Iran to lose weight according to the criteria explained in section 2.2.1. Participants included those who were able to demonstrate that they were able to keep an adequate 4-day food record and readiness to safely participate in daily physical activity.

The study was approved by the Ethical Committee of The Digestive Research Institute**,** Tehran University of Medical Science. All participants provided their signed consent prior to study enrollment. This trial was registered at http:/www.**irct**.ir/ as IRCT201402177754N5.

3.2.2 Design

The study was a 2-arm, randomized clinical trial. Eligible participants were randomly assigned after baseline measures by using a computer-generated random-numbers method by the project coordinator with allocation concealed from the participants and dietitians until randomization was revealed to the study participants at the initial intervention clinic appointment.

The groups were the Water group in which participants replaced habitual intake of DBs with a glass of water (250 ml) after the main meal (lunch) and the DBs group where participants were instructed to drink DBs once a day (250ml), after their main meal (lunch) 5 times a week, and then water only after the main meal (lunch) on the remaining days of the week. Both groups were free to drink water as a beverage during the rest of the days but were not allowed to have DBs consumption. In addition, both groups were asked not to drink DBs or water during the meal and also not add low calorie sweeteners such as aspartame or sucralose to beverages such as tea or coffee.

Bi-weekly visits to the dietitian were required in order to promote adherence to the hypo-energetic diet and beverage substitution. Those in the DBs group were given beverages at their bi-weekly treatment visits. Both groups started a hypo-energetic diet according to the NovinDiet Protocol, which included advice to gradually increase activity levels to achieve 60min of moderate activity 5 d/wk.

3.2.3 Screening visit

At the screening visits participants underwent a physical examination by a General practitioner (see section 2.2.2.1), completed the Beck's Depression Inventory (204) (See Appendix 5), and completed the Physical Activity Readiness Questionnaire (PAR-Q) (206) (See Appendix 4). Potential participants were instructed in how to keep a 4-day food record for 4 consecutive days, which included 3 weekdays and 1 weekend day (see section 2.2.4.2) (See Appendix 2). The food record was evaluated for completeness at the next visit and subjects were excluded if it was not sufficiently detailed.

Two subjects were excluded by Beck's Depression Inventory scores (greater than 9) and 5 subjects were excluded because they produced a food record with insufficient detail.

3.2.4 Dietary and activity intervention sessions

This study was performed in NovinDiet Clinic, Tehran, Iran. NovinDiet Clinic is a private weight loss clinic which uses an integrated approach (dietary, behavioural, exercise and medical treatments) weight loss plan. The NovinDiet Protocol has been described in detail in Section 2.2.5.1.

3.2.5 Measurements

3.2.5.1 Anthropometric measurement

Anthropometric measurements, including weight, height, BMI and waist circumference, were taken at the baseline, 12 weeks and 24 weeks (except height which was taken only at the screening visit), by the dietitian. The details of anthropometric measurements have been described in Section 2.2.2.2.

3.2.5.2 Food intake measurement

To estimate food intake, the participants were asked to record their food intake for 4 consecutive days, which included 3 weekdays and 1 weekend day, while they were undertaking a free life weight loss diet (Section 2.2.6). Energy and macronutrient intake at the baseline and the last week of the intervention (week 24) were analyzed by Nutritionist IV software.

3.2.5.3 Blood Sample measurement

Blood samples were taken after overnight (10- 12 h) fasting by nurse, between 07:00 and 09:00, at the baseline, 12 weeks and 24 weeks for biochemical, cellular and hormonal measurements according to the standard protocol (Section 2.2.3). Blood sample measurements were performed for FPG, 2hpp plasma glucose, serum insulin and HOMA-IR (Section 2.2.3.1), and lipid profiles including the serum total cholesterol (TC), triglyceride (TG), and high-density lipoprotein (HDL) cholesterol (Section 2.2.3.2).

3.2.6 Statistical analyses

3.2.6.1 Power and estimated sample size

The primary outcome addressed in this study was the difference in body weight loss during the 24 week weight loss program. The power calculation was based on that described by Dennis et al (2010) (104) except I chose the power of 0.85 ($\alpha = 0.05$, power = 0.85), with expected differences in weight loss between weight loss diet groups (2.0 ± 2.5 kg) to determine the targeted final sample size ($n = 56$). Considering a dropout rate of 20% the sample size required was 67.

3.2.6.2 Statistical methods

Baseline values of cardiovascular risk factors (including weight, waist circumference, LDL-c, HDL-c, TC, FPG, TG , fasting insulin, HOMA IR, HA1C, 2hpp glucose and food intake data) were compared between the Water and DBs groups using unpaired *t*-tests.

At baseline, distribution was normal for all variables. Repeated-measures two-way analysis of variance (ANOVA) with diet groups (Water or DBs) and time (weeks 0 and 24) as a within-subject factor was used to assess the effects of dietary intervention by comparing changes in the dependent variables between the groups over time. Where there was a significant main effect but no interaction, post-hoc comparisons were performed as appropriate with Bonferroni's adjustment for multiple comparisons to determine differences between group means. Statistical significance was set at $p \leq 0.05$. All data are presented as mean ± SD unless otherwise stated. Associations between variables were assessed by simple correlational analyses (Pearson's *r*). All statistical analyses were performed using SPSS 22.0 for Windows (SPSS Inc., USA).

3.3 Results

3.3.1 Physical characteristics(Baseline characteristics)

Eighty nine obese and overweight participants were recruited and 62 participants completed the 24-week intervention (71% of the original population, **Figure 3.1**). From 89 individuals who were interested in participating in the study, 2 participants were excluded from the study because of the results of Beck depression questionnaire. Five potential participants were excluded because they stopped keeping the dietary record or filled it in insufficiently. Blood test results at baseline revealed that 11 patients were ineligible because of having one or more of the exclusion criteria. The remaining 71 participants gave written consent and then randomly 35 participants were allocated to the water and 36 to the DBs group. After starting the intervention, a total of 9 subjects dropped out because they did not wish to continue or they changed their living places. At Week 12, the retention rates were 97% for DBs group and 91% for Water group, and at Week 24, those rate were 89% for DBs and 86% for Water. As 2 participants in the Water group were eliminated because of one unexpected pregnancy and the other one diagnosed suffering from asthma, it could be said that the retention rates did not differ between groups.

At baseline, there were no statistically significant differences in physical characteristics or biochemical measurements between the intervention groups or between those who completed or did not complete the study once recruited (**Table 3.1**).

3.3.2 Anthropometric measurement

All participants in both groups lost weight during the first 12 weeks of the study and continued to lose weight until the final 24 week point, although at a slower rate (**Figure 3.2**). As shown in **Table 3.2**, there was a significant weight reduction in each group after 24 weeks (DBs=- 7.6 ± 2.1 kg vs. water=-8.8± 1.9kg, time effects, $P<0.001$). As shown in Figure 2, there was a significant group × time interaction for weight loss over 24 weeks ($P=0.015$).

BMI reduction in each group was in the expected direction with significant effects over 24 weeks for both groups (time effects, $P< 0.001$). However, the decline in BMI was larger in the water group than the DBs group after 24 weeks (Water=-3.4±0.7 kg/m^2 vs. DBs=-2.9±0.8 kg/m^2 (**Table 3.2**). There was a significant group × time interaction in BMI reduction over 24 weeks ($P=0.002$).

In both groups, waist circumference had decreased significantly after 24 weeks of intervention (time effects, $P<0.001$). The mean (±SD) waist circumference decline was -8.6 ± 3.4 cm in the Water group, and -7.9 ± 2.7 cm in the DBs group at 24 weeks. However, there was no group × time interaction in the reduction in waist circumference over 24 weeks ($P=0.35$).

3.3.3 Lipid profile

Reductions in total cholesterol, low-density lipoprotein cholesterol, and triglyceride concentration and increase in high density lipoprotein cholesterol were detected over the 24 weeks of study in each group (P<0.001), but there were no significant differences in these results between the groups over 24 weeks (**Table 3.2**).

Total cholesterol after 24 weeks had decreased by -0.53 ± 0.16 mmol/l in the Water group vs. -0.49 ± 0.17 mmol/l in the DBs group (no significant group × time interaction, P= 0.268).

High-density lipoprotein cholesterol (HDL-C) concentration after 24 weeks had increased by 0.125 ± 0.045 mmol/l in the Water group vs. 0.124 ± 0.050 mmol/l in the DBs group (no significant group × time interaction, P= 0.912), whereas low-density lipoprotein cholesterol (LDL-C) had decreased by -0.534 ± 0.164 (Water) and -0.551 ± 0.191 mmol/l (DBs) (no significant group × time interaction, P=0.761).

Triglyceride levels after 24 weeks had decreased by -0.281 ± 0.109 (Water) and -0.280 ± 0.122 mmol/l (DBs) (no significant group × time interaction, P=0.994).

3.3.4 Glucose metabolism measurement

Data analysis showed that fasting plasma glucose, fasting serum insulin, 2 hour postprandial (2hpp) glucose, HbA_{1C} and HOMA-IR all decreased over time in both groups ($P < 0.001$ for time effect of all variables). However, between groups differences were only significant for insulin, 2hpp and HOMA-IR (**Table 3.2**). The average reduction in fasting plasma glucose over 24 weeks, was -0.767 ± 0.278 (Water group) and -0.681 ± 0.169 mmol/l (DBs). However, there was no significant group by time interaction in fasting Plasma glucose reduction over 24 weeks (P=0.143).

In terms of 2hpp differences during the 24 weeks of intervention, the mean reduction of 2hpp was – 1.024 ± 0.254 (Water) and -0.719 ± 0.275 mmol/l (DB). As shown in Table 2, there was a significant group * time interaction in 2hpp glucose effects over 24 weeks (P<0.001).

HbA_{1C} decline over 24 weeks in the Water group was -0.54 ± 0.12 %, while this reduction was -0.47 ± 0.17 % in DBs group at Week 24. But as shown in Table 2, there was no significant difference in the changes between the groups after 24 weeks (P= 0.066).

Fasting serum insulin concentration decreased significantly over time, with differences between the two groups (P<0.001). The reduction of insulin was -2.8±0.8 (Water) and -1.8±1.3 mU/l (DBs) after 24 weeks. Furthermore, there was a significant improvement in insulin resistance in the Water group compared with DBs group over 24 weeks (time×group interaction, P<0.001). HOMA IR decreased by -0.097± 0.049 in the Water compared with -0.057± 0.042 in the DBs group over the 24 weeks (**Table 3.2**). There was a significant group by time interaction (P<0.001).

3.3.5 Food intake measurement

At baseline, there was no significant difference in energy intake. Estimated energy intake showed a significant reduction over time in both groups (P < 0.001 for time effect). Estimated total energy intake indicated a decrease of 586 ± 245 Kcal/ d in the Water group, while this index declined 455 ± 164 Kcal/d in the DBs group over the experiment. As shown in **Table 3.3**, there was a significant group× time interaction for total energy intake over 24 weeks (P=0.015).

In addition, macronutrient intake measurements showed no significant differences between the two groups at baseline. However, there was a greater carbohydrate deficit in the water group compared to the DBs group during the 24 weeks of intervention (group by time interaction, P= 0.01, **Table 3.3**).

3.4 Discussion

The aim of this study was to compare the effects of DBs and water consumption after the lunch as a main meal on weight loss, and also indices of carbohydrate and lipid metabolism in overweight and obese women attending the weight loss program for 24 weeks. The results of this study showed that drinking water may lead to more weight loss and a greater improvement in insulin sensitivity, measured by HOMA IR and 2hpp glucose levels, compared with drinking DBs after the main meal in overweight and obese female adults.

To my knowledge, this is the first randomized controlled trial in which the effect on weight loss of either replacing DBs with water, or continuing to consume DBs, is investigated in overweight and obese women who are regular consumers of DBs with their meal, whilst undertaking a voluntary integrated weight reduction program for 24 weeks. Although some previous intervention studies compared the effects of water consumption on weight loss, their protocols were different. In some studies the effects of either water or DBs consumption in comparison to SSBs without any hypocaloric diet (127) were compared. In other studies the effects of drinking pre-meal water were evaluated during a hypoenergetic diet. In a very recent study (128), drinking water and diet beverage was compared in subjects undergoing cognitive behavior therapy only for a short period of 12 weeks.

All participants in the present integrated weight loss program significantly lost weight in a way consistent with their prescription assignment. In more intensive clinic-based behavioral lifestyle modification programs, 5–10% weight losses have been detected at 6 months (216-218) which were similar to the weight losses observed in the current study. This is not unexpected as the weight loss program includes energy restriction, activity monitoring, and frequent patient visit and consultation in the clinic. It has been shown that these approaches are more consistently effective compared with others recommending small but theoretically sustainable lifestyle modifications that can be made to improve health (219).

Participants in the Water group of the present study consumed 250ml of water after their lunch over 24 weeks and they lost 1.2 kg more (13.6% greater weight loss) than those in DBs group. Stookey and colleagues reported a 2-kg greater weight loss in water consumers (drinking ≥1 l water per day) on a hypoenergetic diet compared with those who consumed less water. However participants in that study were not randomly assigned to drink more water (202). In the study of Dennis et al (96), participants who were randomly assigned to drink pre-meal water lost ~2 kg more weight than participants on the hypoenergetic diet alone. The current study showed a significant interaction between beverage use and time for weight loss, suggesting a benefit from water compared with diet beverage consumption. These results are consistent with the outcome of Dennis's study. However, in the present study, subjects in both groups had either water or DBs after their meal rather than pre-meal which is more representative of normal behavior amongst this group. On the other hand, the present results are contradictory to some other studies which showed either no significant difference in effects on weight loss (127) between water and DBs or reported superior impact on weight loss with DBs compared with water (128). However, it should be noted that these previous studies had different design in terms of not including any weight loss plan (127) or have only cognitive behavioral therapy for weight loss during a short period of 12 weeks (128). Furthermore, the volume of beverage and the time of drinking water and beverage were different in these studies. Future studies could assess whether the amount of water, the timing (i.e. pre-meal consumption compared with during the meal, or after the meal), or other factors could impact on the effects of replacement of diet beverage with water, on weight loss.

In the present study, the effect of substitution of DBs with water on weight loss may lead to better adherence to the weight loss diet in the Water group. The greater weight loss in the Water group might be explained by a greater reduction in energy intake in this group compared with the DBs group. The greater reduction in absolute carbohydrate consumption in the Water group compared with the DBs group might also contribute to the greater weight loss in the Water group. However, finding underlying mechanisms for better weight loss in the Water group compared with the DBs group needs further long term studies.

In the current study, major reductions in waist circumference and significant improvements in cardio metabolic risk characteristics were observed in both groups over 24 weeks, as would be expected given the weight loss observed. Interestingly, despite similar changes in fasting glucose levels in both groups, insulin sensitivity appeared to be improved more with Water group compared with DBs group over the 24 weeks.

These findings may have clinical implications, indicating that drinking only water not DBs could improve weight loss, when people use a weight loss program. Nutritionists and popular weight loss programs usually advise individuals who are keen to lose weight to increase their water consumption (94, 95). On the other hand, most obese people believe that they can drink diet beverages during a low-caloric diet without any deleterious effects on their weight management. It has been claimed, based on the results of previous studies on replacement of energy containing beverages with either water or DBs that both DBs and water would be effective weight-loss strategies. However, the results do not appear to support these claims (127). In the recent study (128) with the opposite results compared with the present study, participants were only on CBT and the intervention period was as short as 12 weeks. In addition, participants had at least 3 diet beverage drinks during the week with no limitation for other beverage consumption, while participants in the present study habitually drank diet beverages prior to the study. Future research is required to examine the longer-term health effects of consuming water as a replacement for DBs in order to establish whether the benefits demonstrated in the present study are sustained.

The principal strength of this study is that it was a randomized, outpatient clinical trial, whilst participants were on a comprehensive diet plan for weight control in a long term intervention (i.e. 24 weeks). Secondly, participants wished to lose weight and were middle-aged overweight and obese women who were able to comply with a weight loss plan, hence they demonstrated that they were motivated to adhere to the weight loss diet protocol (127). Thirdly providing the diet beverages for DBs group and water for the Water group was an incentive for regular by-weekly visits with the dietitian when compliance could be encouraged in both groups.

On the other hand, without long-term follow-up, it is not known whether participants could comply with only water as the beverage, or whether the beneficial effects would be sustained in the longer term. In conclusion, replacing DBs with water consumption would appear to impact beneficially on weight loss and insulin sensitivity in overweight and obese participants adhering to a weight loss diet. Longer term studies are now required, in both healthy overweight and obese patients, and in those whose insulin sensitivity is compromised (e.g. pre diabetic and diabetic patients).

Table 3.1.

subject characteristics before intervention[1]

	water group(n=35)	DBs Group(n=36)
Age, y	32.2± 6.9[2]	31.7 ± 6.8
Body weight, kg	88.2 ± 8.8	87.6 ± 9.8
Height , cm	161.3 ± 4.9	161.7 ± 4.2
BMI , kg/m²	33.9 ± 3	33.5 ± 3.6
Waist Circumference, cm	104.6± 5.8	103.5 ± 5.9
Married	73%	75%
Total cholesterol, mmol/l	4.8± 0.4	4.7± 0.4
HDL cholesterol, mmol/l	1.3± 0.2	1.3± 0.2
LDL cholesterol, mmol/l	2.8± 0.4	2.7± 0.5
Triglycerides, mmol/l	1.7±0.2	1.6 ± 0.2
FPG, mmol/l	5.2±0.5	5.3±0.4
2hpp, mmol/l	6.9 ± 0.7	6.7±0.7
HbA1c,%	5.1 ± 0.4	5 ± 0.4
Insulin, m U/l	13.7 ± 2.1	13.1±3.1
HOMA-IR	3.2±0.6	3.1 ± 0.8

[1]Group difference, $P > 0.05$. There were no significant differences between groups at baseline. DB: diet beverage, FPG: fasting plasma glucose, HbA1c: glycated hemoglobin, 2hpp, 2-h postprandial.

[2]Mean ± SD (all such values).

Table 3.2. Anthropometric and blood measurement characteristics in Water and DBs groups before and after the 24-week interventions[1]

	Water Group(n=30)		DB Group(n=32)		P for time × group[2]
	Baseline	week 24	Baseline	week 24	
Weight, kg	88.7 ± 8.9	79.9 ± 8.3	87.9 ± 9.9	80.3 ± 10.2	0.015
BMI, kg/m²	33.9 ± 3	30.6 ± 2.8	33.5 ± 3.6	30.6 ± 3.8	0.002
Waist circumference, cm[3]	104.9 ± 5.9	96.3 ± 5.7	103.7 ± 6.2	95.8 ± 6.7	0.354
Total cholesterol, mmol/l[3]	4.9 ± 0.4	4.3 ± 0.4	4.7 ± 0.5	4.2 ± 0.5	0.268
HDL-C, mmol/l[3]	1.3 ± 0.2	1.4 ± 0.2	1.3 ±0.2	1.4 ± 0.2	0.912
LDL-C, mmol/l[3]	2.8 ± 0.4	2.1 ± 0.3	2.7 ± 0.5	2.7 ± 0.5	0.761
Triglycerides, mmol/l[3]	1.7 ± 0.2	1.4± 0.2	1.6 ± 0.2	1.3± 0.1	0.994
FPG, mmol/l	5.3 ± 0.5	4.5 ± 0.5	5.3 ± 0.4	4.6 ± 0.4	0.143
2hpp, mmol/l	6.7 ± 0.7	5.7 ± 0.5	6.6 ± 0.6	5.9 ± 0.6	<0.001
HbA1c,%[3]	5.1 ± 0.4	4.5 ± 0.5	4.9 ± 0.4	4.5 ± 0.4	0.066
Insulin, m U/l	13.6 ± 2.2	10.8 ± 1.9	13 ± 3.3	11.3 ± 2.8	<0.001
HOMA-IR	3.2 ± 0.7	2.2 ± 0.5	3.1 ± 0.8	2.3 ± 0.7	<0.001

[1] Data are presented as mean ± SD for the 62 participants who completed the study

[2] P values are for Water relative to DBs group (time × Group interaction) by repeated-measures two way ANOVA

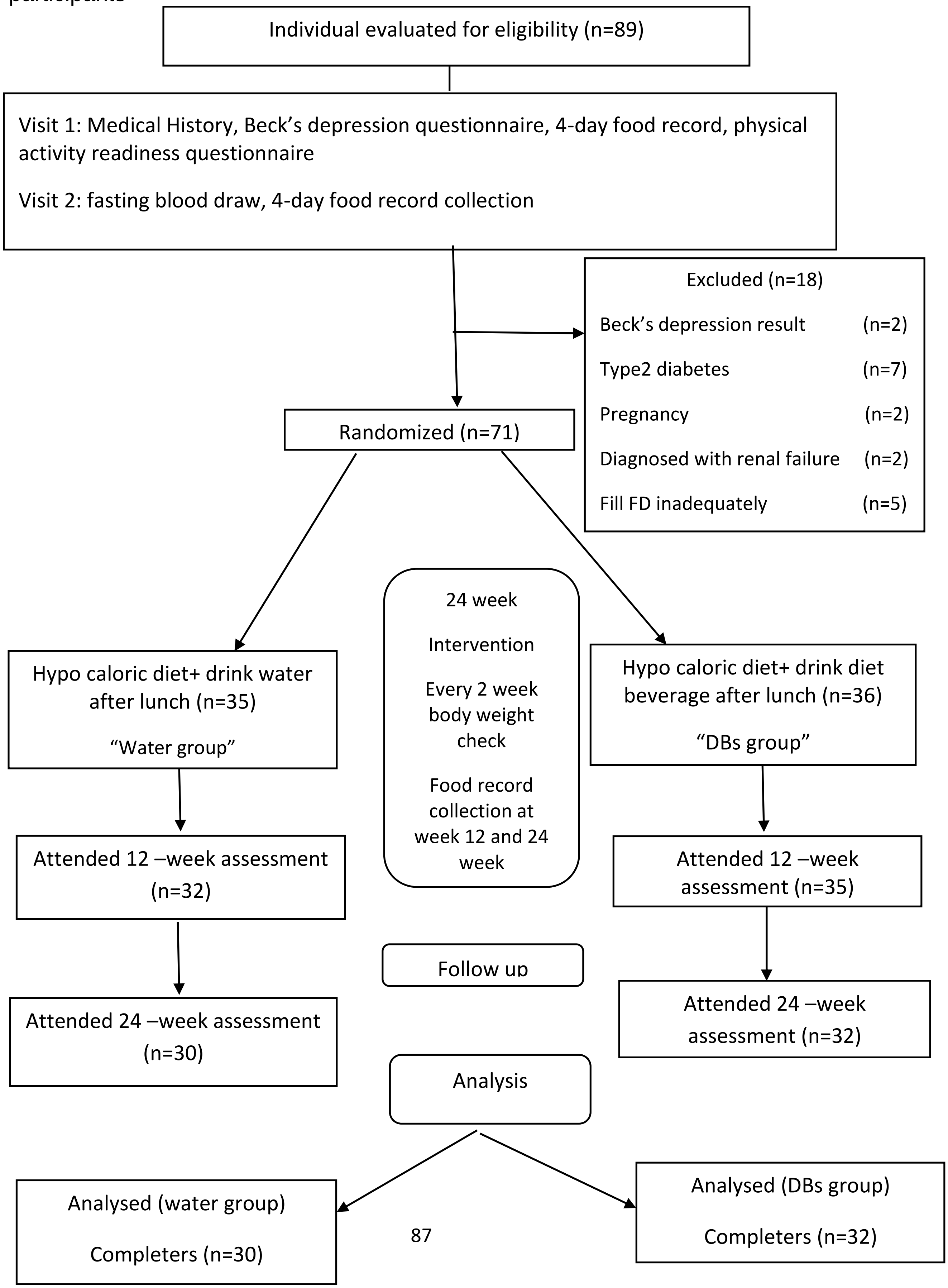
[3] Significant main effect of time, $P < 0.001$

Table 3.3. Self- reported dietary intake in Water and DBs Groups before and after the 24-week interventions[1]

Intake	Water Group(n=30)		DBs Group(n=32)		P for time × group[2]
	Baseline	week 24	Baseline	week 24	
Total Energy (kcal)	2457±303	1871±203	2438±295	1984±348	0.015
Protein (g)	90.2±7.3	79.6±8.7	87.5±7.6	76.9±11.3	0.92
Protein (%)	14.9%±2.2	17.1%±1.6	14.5%±2.1	15.7%±1.8	
Fat (g)	96.5±18.4	66.8±9.8	95.1±19.5	68.4±15.4	0.39
Fat (%)	35.1%±3.1	32%±2.6	34.8%±3.9	30.8%±2.1	
Carbohydrate (g)	306.8±37.4	237.9±28.7	308.2±36.5	265.2±45.3	0.001
Carbohydrate (%)	50%±1.7	50.9%±2.5	50.6%±2.7	53.5%±1.7	
Fibre(g)	20.6±5.5	22±5.6	20.75±3.2	22.14±3.24	0.45

[1] Data are presented as mean ± SD for the 62 participants who completed the study
[2] P values are for Water relative to DBs group (time × Group interaction) by repeated-measures two way ANOVA

Figure 3.1. Screening, enrolment, randomization and follow up of study participants



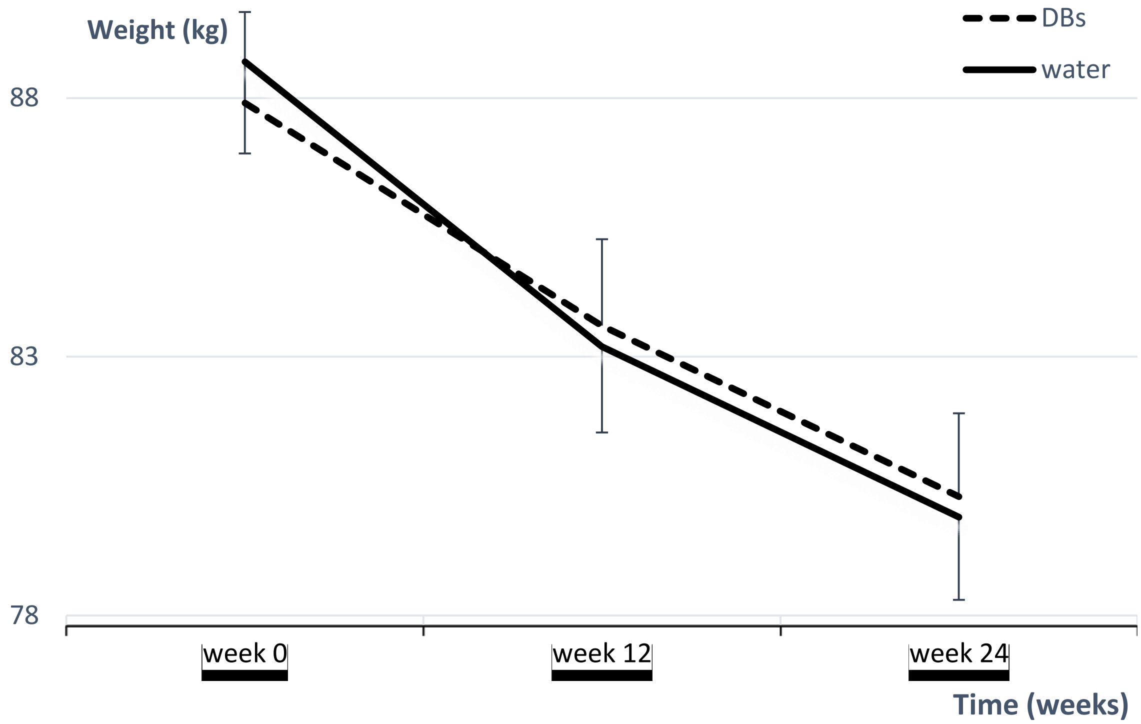


Figure 3.2 Mean (±SEM) body weight before and after 24 wk of energy restriction with either drinking water (Water; *n* = 30) or diet beverages (DBs; *n* = 32) in the participants who completed the study.
$P < 0.001$ for the main effect of time, and $P = 0.015$ for the time × group interaction (repeated-measures ANOVA).

Chapter 4.Effects of replacing diet beverages with water on weight loss and weight maintenance: 18 month follow up, randomized clinical trial

4.1 Introduction

The results of the first study (220) presented in Chapter 3, which was the first phase of the present longitudinal study, showed that replacement of DBs with water may introduce a greater weight loss compared with DBs in obese women being on a weight loss program for 24 weeks. However, as with all weight loss interventions, the long term effectiveness of such an approach is an issue, as someone who is prone to over eating foods may fail to maintain their changed behavior in the long term and regain their weight.

The present study aimed to follow up the participants from the first study (DBs study), in order to investigate the changes in weight and anthropometric measurements after a total of 18 months, consisting of 24 weeks active weight loss and 53 weeks weight maintenance. The secondary objectives were to compare the biochemical indicators of cardiometabolic risk during this period.

4.2 Methods

4.2.1 Subjects

Eighty nine healthy overweight and obese females were selected between March 2014 and June 2014 (participants of the study in chapter 3) from participants attending NovinDiet Clinic, Tehran, Iran to lose weight according to the criteria explained in section 2.2.1. From 89 participants enrolled in the study, 18 withdrew from the trial prior to the start of study and 71 participants began treatment.

4.2.2 Design

This study was an 18 month 2-arm, randomized clinical trial comparing effects of replacing diet beverages with water as part of a 24 week weight loss program followed by 53 weeks weight maintenance program. Eligible participants were randomly allocated after the baseline measures to the two groups which were explained in section3.2.2.

The study protocol included a preplanned data analysis with the primary result of weight loss at 24 weeks (weight loss plan) and after 77 weeks (53 week follow up weight maintenance plan).The intervention groups were the water group and the DBs group as explained in section 3.2.2.

4.2.3 Dietary and activity intervention sessions

4.2.3.1 Weight loss period

This study performed in NovinDiet Clinic, Tehran, Iran, as described in chapter 3. The NovinDiet's Protocol for weight loss is described in detail in Section 2.2.5.1.

4.2.3.2 Weight loss maintenance Period

During the 53 weeks weight maintenance program, participants attended a 60-min group session, every 4 weeks, led by a registered dietitian to encourage energy restriction and moderate-intensity physical activity. The participants were classified by treatment group when attending the group session. At the same time, all participants had then regular individual visits every month for general nutrition consultation in order to know how they could manage their weight maintenance and were also weighed at these visits. Adherence to the intervention was assessed by the dietitians, based on food, beverage, and physical activity logs as well as checking their weight.

Both groups' participants were provided with the same program and their only difference was in terms of informing them about the type of beverages they should consume.

4.2.4 Measurements

4.2.4.1 Anthropometric measurement

All anthropometric measurements of participants were conducted at baseline, 24 wk, and 77wk (except height, which was taken only at the screening visit) by the dietitian. The details of anthropometric measurements have been described in Section 2.2.2.2.

4.2.4.2 Food intake measurement

Participants completed the food diary record at baseline, 24 and 77 weeks to assess the energy and macronutrient intakes which was undertaken by using Nutritionist IV software (version 4.1; Hearst). Adherence to beverages treatment was determined from daily beverage logs collected at each visit.

To estimate food intake, the participants were asked to record their food intake for 4 consecutive days, which included 3 weekdays and 1 weekend day, while they were undertaking a free life weight loss diet (Section 2.2.6).

4.2.4.3 Blood Sample measurement

Blood samples were taken after overnight (10- 12 h) fasting, between 07:00 and 09:00, at the baseline, 24 weeks and 77 weeks for biochemical, cellular and hormonal measurements according to the standard protocol (Section 2.2.3). Blood sample measurements were performed for FPG, 2hpp plasma glucose, serum insulin and HOMA-IR (Section 2.2.3.1), and lipid profiles including the serum total cholesterol (TC), triglyceride (TG), and high-density lipoprotein (HDL) cholesterol (Section 2.2.3.2).

The details of the anthropometric measures and blood sample measurements have been described previously in chapter 2(section 2.2.2 - 2.2.5).

4.2.5 Statistical analyses

Baseline values of cardiovascular disease risk factors (including weight, WC, LDL cholesterol, HDL cholesterol, TC, FPG, triglycerides, fasting insulin, HOMA-IR, HbA1c, 2hpp glucose) and food intake data were compared within the water and DBs groups by using unpaired t tests.

At baseline, distribution was normal for all variables. Repeated measures 2-factor ANOVA with diet groups (water or DBs) and time (weeks 0, 24 and 77) as a within-subject factor was used to assess the effects of the dietary intervention by comparing changes in the dependent variables between the groups over time. Where there was a significant main effect but no interaction, post hoc comparisons were performed as appropriate with Bonferroni's adjustment for multiple comparisons to determine differences between group means.

Statistical significance was set at $P \leq 0.05$. All data are presented as means ± SDs unless otherwise indicated. Associations between variables were assessed by simple correlational analyses (Pearson's r). All statistical analyses were performed by using SPSS 22.0 for Windows (SPSS Inc.).

The primary result addressed in this study was the difference in body weight loss during the 18 month of study. With the assumption of a detectable difference in weight loss of 2.5 kg between the 2 groups at 18 month and an SD of 3 kg, 51 participants were required to achieve 85% power of detecting a treatment effect (2-sided significance level of 5%). Allowing for a dropout rate of 25%, for 18 month intervention, the sample size required was 70.

4.3 Results

4.3.1 Physical characteristics

From 71 participants who began the study, 56 (or 79% of) participants completed the 18 month trial (**Figure 4.1**). From week 24 to week 77, a total of six participants dropped out. Three potential participants withdrew from the water group (1 without any reason, 1 because of time constraint and 1 for dissatisfaction with program). Also in the DBs group 3 participants withdrew (1 because of relocation, 1 left because need for urgent surgery, 1 because of a scheduling conflict).

At baseline, there were no statistically significant differences in physical characteristics or biochemical measurements between the intervention groups or between those who completed or did not complete the study once recruited (**Table 4.1**).

Compliance with consumption of prescribed beverages at the end of trial was high according to the beverages log - being 99.2% for the water and 98.7 for the DBs group.

4.3.2 Body weight, BMI, and WC

As shown in **Table 4.2**, there was a significant difference in weight reduction between the two groups after 18 months ($P=0.005$). The final weight change (means ± SDs) was -10.3±3.8kg in the water group and -7.8±3.4kg in DBs group (Time effects, $P< 0.001$).

BMI reduction in each group was significant over 18 months for both groups ($P< 0.001$). However, the decline in BMI was greater in the water group than the in DBs group after 18 months (water: -3.9± 1.3kg/m^2; DBs:-3.0±1.3 kg/m^2). There was a significant group *time interaction in BMI reduction over 18 months ($P=0.005$).

In both groups, waist circumference had decreased after 18 months of intervention (Water:-10.4±4.6 cm; DBs:-9±3.8cm, P<0.001) with no significant group * time interaction in WC effects between the two groups after 18 months of trial (P=0.286).

4.3.3 Lipid profile

Reductions in TC, LDL cholesterol, and triglyceride concentration and an increase in HDL cholesterol were detected over the 18 month trial in each group (P < 0.001), but there were no significant differences in these results between the two groups (**Table 4.2**).

TC after 18 months had decreased after 18 months of intervention (Water: -0.5±0.3 mmol/L; DBs: -0.5±0.2 mmol/L) with no significant group * time interaction in TC effects between the two groups after 18 months of trial (P=0.548).

HDL cholesterol concentration after 18 months had increased equally by 0.2±0.1 mmol/L in both groups (no significant group * time interaction, P = 0.894).

LDL cholesterol had decreased after 18 months of intervention (Water: -0.5±0.4 mmol/L; DBs: -0.6±0.3 mmol/L) with no significant group * time interaction in LDL cholesterol effects between the two groups after 18 months of trial (P=0.787).

Triglyceride concentrations after 18 months had decreased (Water: -0.3±0.1mmol/L; DBs: 0.3±0.1mmol/L) with no significant group * time interaction in LDL cholesterol effects between the two groups after 18 months of trial (P=0.273).

4.3.4 Glucose metabolism measurement

Fasting plasma glucose, fasting serum insulin, HbA1c and HOMA-IR, and 2 hour postprandial (2hpp) glucose, all decreased over time in both groups (*P* < 0.001). Also between group differences were significant for all, except fasting plasma glucose after the 18 month intervention (**Table 4.2**).

The mean reduction in fasting plasma glucose over the intervention was -0.9±0.5 mmol/L (water) and -0.7±0.3 mmol/L (DBs). However, there was no significant group*time interaction in fasting glucose decrease over 18 month follow-up (0.156).

There was a significant difference in fasting serum insulin concentration between the two groups over the study (**Table 4.2**) ($P<0.001$).The reduction of insulin was -3.5±1.8 mU/L in the water group and -1.7±2.1 mU/L in the DBs group over 18 months.

HbA1c decline over 18 months of intervention in the water group was -0.7% ±0.3%, whereas this reduction was -0.5% ±0.3% in the DBs group at 18 months. There was a significant difference in the changes between the groups over 18 months ($P=0.030$).

In terms of 2hpp differences during the 18 months of trial, the mean reduction was −1.4±0.6mmol/L (water) and −0.9±0.5mmol/L (DBs) which was a significant group*time interaction in 2hpp glucose effects over 18 months ($P<0.001$).

HOMA-IR decreased by −1.2±0.6 in the water group compared with −0.7±0.6 in the DBs group over 18 months (**Table 4.2**). There was a significant group × time interaction ($P<0.001$)

4.3.5 Food intake measurement

As reported at baseline, there was no significant difference in energy intake. After 18 months, estimated energy intake measurements showed a significant reduction over time in both groups ($P <0.001$ for time effect). As presented in **Table 4.3**, there was a significant group*time interaction for total energy intake over 18 months ($P = 0.001$).

Besides, macronutrient intake measurements indicated no significant differences between the 2 groups at baseline. Nevertheless, there was greater carbohydrate reduction in the water group than in the DBs group during 18 months (group*time interaction, $P<0.001$, **Table 4.3**).

4.4 Discussion

The present 18 month clinical trial indicates the beneficial effect of replacing DBs with water, in regular consumer of DBs who were overweight and obese women, while attending a 24 weeks voluntary integrated weight-reduction program (220), followed by a 53 week weight maintenance program.

To our knowledge, the present 18 month randomized clinical trial is the longest study up to now to compare the effects of DBs and water consumption after the lunch on weight loss, weight maintenance and also indices of carbohydrate and lipid metabolism attending the weight control program.

The present trial provides evidence that drinking water may lead to more weight loss and a greater improvement in insulin sensitivity than drinking DBs. This is in contrast to recent studies which claimed a favorable impact of DBs in weight loss and weight maintenance (127, 128, 203). However, the present results are consistent with the study by Dennis et al, which showed the positive effects of drinking pre-meal water on weight loss during a hypoenergetic diet (96). In addition, some observational studies reported positive associations between DBs, obesity and weight gain (200-222).

The weight loss and weight maintenance of participants in our integrated program was consistent with the targets that they were set. 5–10% weight losses have been reported in more intensive clinic-based behavioral lifestyle modification plans (216, 217), which are similar to the weight losses that we observed during the first 6 months of our weight loss plan. This was expected as our weight-loss program contains energy restriction, activity monitoring, and frequent patient visits and consultation in the clinic (220). Successful weight loss interventions are those which produce ≥5% weight loss from baseline followed by a maintenance period in which the weight loss is maintained until at least 12 months from the start (223). The equivalent combination of energy and fat restriction, regular physical activity, and behavioral strategies was also essential for successful weight maintenance (223). Subjects in the water group of our study lost 1.2 kg more (13.6% greater weight loss) than those in the DB group during the first phase of the intervention (i.e. 24 weeks) (220).

In addition, the result of our whole 18 month follow-up corresponds with these definitions of successful weight loss and maintenance plans, as showed that the participants in the water group lost 2.5kg more weight than participants in the DB group.

However, our results are conflicting with the result of Tate et al. study which showed no significant difference in weight loss between water and DBs (127) and another recent intervention study reporting a greater weight loss with DBs compared with water during 12 weeks which was maintained at the follow-up to 1 year (128, 203). However, it should be mentioned that these studies had a different design in terms of not including any weight-loss plan (127) or had only cognitive behavioral therapy for weight loss (128, 203). Moreover, in these studies the size of beverage and the time of drinking (for example consumption before the meal compared with during or after the meal) were different. So, further studies are required to see if those factors could influence the effects of replacement of DBs with water on weight loss.

The beneficial effect of replacement of DBs with water on weight loss in the present study may reflect better adherence to the multidisciplinary weight loss plan in the water group leading to a greater reduction in energy intake, which is consistent with previous findings (198). Also, the greater reduction in total carbohydrate intake seen in the water group compared to the DB group over 18 months of the intervention would also contribute to the greater weight loss in the water group. The present study is also consistent with the suggestion that the use of non- nutrient sweetener within DBs may be associated with the sustained consumption of sweet foods or high-energy foods compared to drinking water (200).

A recent prospective cohort study showed a prominent, positive dose-response relationship between increasing diet beverage intake and increasing abdominal obesity (224). The current study revealed major reductions in WC and significant improvements in cardiometabolic risk characteristics in both groups which could be expected according to the detected weight loss during this time.

However, there were no differences between the 2 groups. By contrast, despite the similar changes in fasting glucose concentrations in both groups, insulin sensitivity improved more in the water group than the DBs group over the study.

The findings of this study may have clinical implications which indicate drinking only water could improve weight loss and maintain it for the long term. It is in agreement with nutritionists and popular weight-loss programs which recommend an increase in water consumption for losing weight (94, 95). In contrast, many obese or overweight persons assume they can continue drinking DBs when they wish to lose weight or maintain their weight loss without any detrimental effects on their weight management. Replacing DBs with water consumption also appeared to have a beneficial impact on the improvement in insulin sensitivity during weight loss and the subsequent weight maintenance period.

The main strength of this study was the fact that it was a randomized, outpatient clinical trial with a comprehensive management plan for an 18 month intervention. All participants wished to lose weight and the middle-aged overweight and obese women who participated were able to follow the weight-management plan and adhere to the weight-management protocol (225). Finally, providing the diet beverages for the DBs group and water for the water group at each visit was an incentive for regular visits to see the dietitian. This enabled compliance to be reinforced in both study groups. On the other hand, the limitation of this study is that only female adults who were overweight or obese and were regular DBs users were studied, rather than a wider range of participants, such as People used to drink SSBs which may have different results. In addition, finding the actual mechanisms behind the results seen in the present study needs further research.

Table 4.1

Subject characteristics before the intervention[1]

	water group(n=35)	DBs Group(n=36)
Age, y	32.2± 6.9[2]	31.7 ± 6.8
Body weight, kg	88.2 ± 8.8	87.6 ± 9.8
BMI, kg/m²	33.9 ± 3	33.5 ± 3.6
WC, cm	104.6± 5.8	103.5 ± 5.9
TC, mmol/l	4.8± 0.4	4.7± 0.4
HDL-C, mmol/l	1.3± 0.2	1.3± 0.2
LDL-C, mmol/l	2.8± 0.4	2.7± 0.5
TG, mmol/l	1.7±0.2	1.6 ± 0.2
FPG, mmol/l	5.2±0.5	5.3±0.4
2hpp, mmol/l	6.9 ± 0.7	6.7±0.7
HbA1c,%	5.1 ± 0.4	5 ± 0.4
Insulin, m U/l	13.7 ± 2.1	13.1±3.1
HOMA-IR	3.2±0.6	3.1 ± 0.8

[1]Group difference, P> 0.05. There were no significant differences between groups at baseline

[2]Mean ± SD (all such values).

Diet beverages: DBs, Waist circumference: circumference: WC, Total cholesterol: TC, Triglyceride: TG, Fasting plasma glucose: FPG , Glycated hemoglobin: Hb A1C, 2 hour post prandial: 2hpp,Homeostasis model assessment of insulin resistance: HOMA-IR

Table 4.2 Anthropometric and blood measurement characteristics in Water and DBs Groups from baseline to week 77

	Water Group(n=35)			DBs Group(n=36)			P for time ×group†
	Baseline	week 24	week 77	Baseline	week 24	week 77	
Weight, kg	88.2(8.8)	79.7(7.9)	78 (6.1)	87.6 (9.8)	80(9.7)	79.8(8.2)	0.005
BMI, kg/m²	33.9 (3)	30.6(2.7)	30(2.3)	33.5(3.6)	30.6(3.5)	30.5(2.9)	0.005
WC, cm ‡	104.6(5.8)	96.4(5.6)	94.3(3.9)	103.5(5.9)	95.8(6.7)	94.5(4.7)	0.286
TC, mmol/l ‡	4.8(0.4)	4.4 ± 0.4	4.3(0.3)	4.7 (0.5)	4.2 (0.4)	4.1 (0.4)	0.548
HDL-C, mmol/l ‡	1.3(0.2)	1.4 (0.2)	1.4(0.2)	1.3 (0.2)	1.4 (0.2)	1.4 (0.1)	0.894
LDL-C, mmol/l ‡	2.8(0.4)	2.3(0.3)	2.2(0.3)	2.7 (0.5)	2.2(0.5)	2.1(0.4)	0.787
TG, mmol/l ‡	1.7(0.2)	1.4(0.2)	1.3(0.2)	1.6 (0.2)	1.3(0.1)	1.3(0.1)	0.273
FPG, mmol/l	5.2(0.5)	4.6(0.5)	4.4(0.4)	5.3 (0.4)	4.7(0.4)	4.6(0.3)	0.156
2hpp, mmol/l	6.9 (0.7)	5.7 (0.5)	5.5(0.4)	6.7 (0.8)	5.9 (0.6)	5.9(0.4)	<0.001
HbA1c,% ‡	5.1 (0.4)	4.5 (0.5)	4.4(0.4)	5 (0.4)	4.5(0.4)	4.4(0.4)	0.0.30
Insulin, m U/l	13.7 (2.1)	10.7(2)	10.2(2)	13.1 (3.1)	11.4(2.9)	11.4(2.5)	<0.001
HOMA-IR	3.2(0.6)	2.2 (0.5)	2(0.5)	3.1 (0.8)	2.4(0.7)	2.3(0.6)	<0.001

* Data are presented as mean (SD) for the 71 participants

† P values are for Water relative to DBs group (time × Group interaction) by repeated measures 2-factor ANOVA.

‡ Significant main effect of time, P < 0.001

Diet beverages: DBs, Waist circumference :WC, Total cholesterol: TC, Triglyceride: TG, Fasting plasma glucose: FPG

Table 4.3 Self-reported dietary intake in Water and DBs Groups before and after the one year follow up of the intervention[1]							
Intake	**Water Group(n=35)**			**DBs Group(n=36)**			**P for time × group[2]**
	Baseline	**week 24**	**week 77**	**Baseline**	**week 24**	**week 77**	
Total Energy (kcal)	2461(300)	1873(192)	1761(125)	2429(300)	1986(355)	1935(224)	0.001
Protein (g)	89.3(7.2)	79.7(8.3)	81.2(1.7)	87.2(7.3)	76.4(11.6)	77.2(8.7)	0.645
Protein (%)	14.7%(2.1)	17.1%(1.6)	18.5(1.7)	14.6%(2)	15.5%(1.6)	16%(1.1)	
Fat (g)	97.4(17.8)	67.4(9.3)	62.9(5.4)	94.3(19.9)	68.5(15.5)	66.1(9.9)	0.113
Fat (%)	35.4%(3)	32.3%(2.3)	31.1%(1.7)	34.6%(4)	30.8%(2.2)	30.7%(1.6)	
Carbohydrate (g)	306.9(37.9)	237(27.6)	217.6(19.5)	307.9(36.3)	266(46.3)	257.7(28.5)	<0.001
Carbohydrate (%)	49.9%(1.7)	50.6%(2.5)	49.4%(2.3)	50.8%(2.7)	50.8%(2.7)	53.3%(1.7)	
Fibre(g)	20.4(5.5)	21.8(5.5)	21.4(4.1)	20.7(3.1)	22.1(3.1)	22.5(2.7)	0.109

[1] Data are presented as mean (SD) for the 71 participants

[2] P values are for Water relative to DBs group (time × Group interaction) by repeated-measures two way ANOVA

DBs: diet beverages

Figure 4.1 Screening, enrollment, randomization and follow up of study participants

Chapter 5. Beneficial effects of replacing diet beverages with water on Type 2 diabetic obese women following a hypo-energetic diet - a randomized, 24 week clinical trial

5.1 Introduction

In chapter 3, the study on the effect of replacing DBs with water, on promoting weight reduction in obese adults without diabetes who were on a hypo-energetic diet was reported (237). The results of this study showed that the Water group had a greater decrease in weight, and insulin resistance over the 24 weeks of study compared with the DBs group. Due to beneficial effects of substitution of DBs with water in overweight/ obese women not only on weight loss but also on insulin sensitivity, it would be interesting to repeat this protocol in those with Type 2 diabetes.

Thus, the purpose of this study was to investigate the effects of replacing DB consumption with water during a comprehensive 24-wk weight-loss program on body weight and abdominal adiposity as a primary outcome, as well as carbohydrate and lipid metabolism as secondary outcomes, in overweight and obese women with Type 2 diabetes.

5.2 Methods

5.2.1 Subjects

124 Obese female adults with diabetes ,$6.5<Hb_{A1C}<7.2$ and only taking Metformin to control their diabetes, were selected between April 2015 and June 2015 from the participants attending NovinDiet Clinic, Tehran, Iran to lose weight according to the criteria explained in section 2.2.1.

Participants included those who were able to demonstrate that they were able to keep an adequate 4- day food record and readiness to safely participate in daily physical activity.

The study was approved by the Ethical Committee of The Digestive Research Institute, Tehran University of Medical Science. All participants provided their signed consent prior to study enrollment. This trial was registered at http:/www.clinical trials.gov/ as NCT02412774.

5.2.2 Design

The study was a 2-arm, single-blind, randomized clinical trial. Eligible participants were randomly allocated after baseline measures to the two groups which explained in section3.2.2.

Eighty-one participants who were eligible for the study were randomly assigned to one of the 2 groups. All had a 2-wk washout period from any artificial sweetener products including diet beverages before intervention.

The intervention groups were the water group and the DBs group as explained in section 3.2.2. To control the effects of menstrual cycle on measurements, participants started the study at the same phase of their menstrual cycle. Bi-weekly visits to the dietitian were required in order to promote adherence to the hypo-energetic diet and beverage substitution.

5.2.3 Screening visit

At the screening visits subjects underwent a physical examination by a General Medicine doctor (see section 2.2.2.1), completed the Beck's Depression Inventory (201) (See Appendix 5), and completed the Physical Activity Readiness Questionnaire (PAR-Q) (202), (See Appendix 4). Potential subjects were instructed in how to keep a 4-day food record for 4 consecutive days which included 3 weekdays and 1 weekend day (see section 2.2.4.2) (See Appendix 2). The food record was evaluated for completeness at the next visit and subjects were excluded if it was not sufficiently detailed.

Four subjects were excluded by Beck's Depression Inventory scores (greater than 9) and three subjects were excluded because they produced a food record with insufficient detail.

5.2.4 Dietary and activity intervention sessions

This study was performed in NovinDiet Clinic, Tehran, Iran. NovinDiet Clinic is a private weight loss clinic which uses an integrated approach (dietary, behavioural, exercise and medical treatments) weight loss plan. The NovinDiet Protocol has been described in details in Section 2.2.5.1

5.2.5 Measurements

5.2.5.1 Anthropometric measurement

Anthropometric measurements, including weight, height, BMI and waist circumference) were taken at the baseline, 12 weeks and 24 weeks (except height which was taken only at the screening visit), by the dietitian. The details of anthropometric measurements have been described in Section 2.2.2.2.

5.2.5.2 Food intake measurement

To estimate food intake, the participants were asked to record their food eaten for 4 consecutive days, which included 3 weekdays and 1 weekend day, while they were in a free life weight loss diet (Section 2.2.6). Energy and macronutrient intake at the baseline and the last week of the intervention (week 24) was analyzed by Nutritionist IV software.

5.2.5.3 Blood Sample measurement

Blood samples were taken after overnight (10- 12 h) fasting, between 07:00 and 09:00, at the baseline, 12 weeks and 24 weeks for biochemical, cellular and hormonal measurements according to the standard protocol (Section 2.2.3). Blood sample measurements were performed for FPG, 2hpp plasma glucose, serum insulin and HOMA-IR (Section 2.2.3.1), and lipid profiles including the serum total cholesterol (TC), triglyceride (TG), and high-density lipoprotein (HDL) cholesterol (Section 2.2.3.2).

5.2.6 Statistical analyses

5.2.6.1 Power and estimated sample size

The primary outcome addressed in this study was the difference in body weight loss during the 24 week weight loss program. The power calculation was based on the previous studies (96, 220) (α = 0.05, power = 0.85), which were performed based upon expected differences in weight loss between weight loss diet groups (2.0 ± 2.5 kg) to determine the targeted final sample size (*n* = 56). Anticipating a dropout rate of 30% the sample size required was 80.

5.2.6.2 Statistical methods

Baseline values of cardiovascular risk factors (including weight, waist circumference, LDL-c, HDL-c, TC, FPG, TG, fasting insulin, HOMA IR, Hb_{A1C}, 2hpp glucose data) were compared between the Water and DBs groups using unpaired *t*-tests.

At baseline, distribution was normal for all variables. All participants who were randomly assigned and completed an initial assessment were included in the final results by using an intention-to-treat analysis. Multiple imputations with the use of linear regression were used to impute missing values from 24 wk and were based on the assumption that data were missing at random.

The primary analysis was an intent to treat linear mixed effect, which assessed at 12 and 24 weeks. These models, which included time, treatment, a time by group interaction and the respective baseline value as principal explanatory variables for all 81 participants.

The per-protocol analysis was also done for the outcomes. The results from per-protocol analysis were also similar to those of the intent to treat analysis in direction and significance. Statistical significance was set at $p \leq 0.05$. All data are presented as mean ± SD unless otherwise stated. Associations between variables were assessed by simple correlational analyses (Pearson's *r*). All statistical analyses were performed using SPSS 22.0 for Windows (SPSS Inc., USA).

5.3 Results

5.3.1 Physical characteristics (Baseline characteristics)

124 female patients with Type 2 diabetes, who believed that they were eligible and expressed an interest in participating in the study, were evaluated for eligibility by a physician. After evaluation, 81 subjects were recruited and 65 subjects completed the 24-week intervention (with 80% retention rate, **Figure 5.1**). 4 subjects were excluded from the study because of the results of Beck depression questionnaire. Three potential subjects were excluded because they stopped keeping the dietary record or filled it in insufficiently. Blood test results at baseline revealed that 20 patients were ineligible because of having one or more of the exclusion criteria. The BMI of 11 subjects were out of range and 5 subjects were not within the age range. The remaining 81 subjects gave written consent and then randomly 41 subjects were allocated to the water and 40 to the DBs group. After starting the intervention, a total of 11 subjects dropped out because they did not wish to continue or they moved away from the area. 2 Participants left the study as they became pregnant. The remaining 3 subjects did not give any reason for their withdrawal.

At baseline, there were no statistically significant differences in age, physical characteristics or biochemical measurements between the groups or between those who completed or did not complete the study once recruited (**Table 5.1**).

5.3.2 Anthropometric measurement

All subjects in both groups lost weight during the first 12 weeks of the study and continued to lose weight until the final 24 week point (**Figure 5.2**). As shown in **Table 5.2**, there was a significant weight reduction in each group after 24 weeks (DBs= -5.25 ± 1.60 kg vs. water= -6.40 ± 2.42 kg, time effects, P<0.001). As shown in Figure 4.2, there was a significant group * time interaction for weight loss over 24 weeks (P=0.006, **Figure 5.2**).

BMI reduction in each group was in the expected direction with significant effects over 24 weeks for both groups (time effects, P< 0.001). However, the decline in BMI was larger in the water group than the DBs group after 24 weeks (DBs= -2.06 ± 0.62 kg/m² vs. Water= -2.49 ± 0.92 kg/m² (**Table 5.2**). The decline in BMI was greater in the water group than the DBs group after 24 weeks (P=0.006).

In both groups, waist circumference had decreased significantly after 24 weeks of intervention (time effects, P<0.001). The mean ± SD waist circumference decline was -6.59 ± 2.57 cm in the Water group and -5.68 ± 1.79 cm in the DBs group at 24 weeks (**Table 5.2**). There was no significant difference in WC effects between the two groups after the intervention (P=0.833).

5.3.3 Lipid profile

Reductions in total cholesterol, low-density lipoprotein cholesterol, and triglyceride concentration and increase in high density lipoprotein cholesterol were detected over the 24 weeks of study in each group (P<0.001). There were no significant differences in LDL-C, HDL-C, TG and TC results between the groups over 24 weeks (**Table 5.2**).

Total cholesterol after 24 weeks had decreased by-0.44 ± 0.14 mmol/l in the Water group vs. -0.50 ± 0.18 mmol/l in the DBs group (P= 0.119) (**Table 5.2**).

Triglyceride levels after 24 weeks had decreased by -0.39 ± 0.08 (Water) and -0.35 ± 0.11 mmol/l (DBs) (P=0.639) (**Table 5.2**).

High-density lipoprotein cholesterol (HDL-C) concentration after 24 weeks had increased by 0.20 ± 0.09mmol/l in the Water group vs. 0.20 ± 0.06mmol/l in the DBs group (P= 0.319), whereas low-density lipoprotein cholesterol (LDL-C) had decreased by -0.51 ± 0.18 (Water) and -0.47 ± 0.17mmol/l (DBs) (P=0.07) (**Table 5.2**).

5.3.4 Glucose metabolism measurement

Data analysis showed that fasting plasma glucose, fasting serum insulin, 2 hour postprandial (2hpp) glucose, HbA_{1C} and HOMA-IR all decreased over time in both groups ($P < 0.001$ for time effect of all variables). Also between group differences were significant for all variables (**Table 5.2**). The mean reduction in fasting plasma glucose over 24 weeks, was -1.63 ± 0.54 (Water group) and -1.29 ± 0.48 mmol/l (DBs)(P=0.005).

In terms of 2hpp changes during the 24 weeks of intervention, the mean reduction was -1.67 ± 0.62 (Water) and -1.35 ± 0.39 mmol/l (DB) (P=0.027) (**Table 5.2**).

HbA_{1C} decline over 24 weeks in the Water group was -1.16 ± 1.09%, while this reduction was -0.42 ± 0.21% in DBs group at Week 24 (P=0.149).

Fasting serum insulin concentration decreased significantly over time, with differences between the two groups (P<0.001). The reduction of insulin was -5.71 ± 2.30 (Water) and -4.16 ± 1.74 mU/l

(DBs) after 24 weeks. Furthermore, there was a significant improvement in insulin resistance, measured by HOMA, in the Water group compared with DBs group over 24 weeks, P=0.011) (**Table 5.2**).

HOMA IR decreased by -3.20 ± 1.17 in the Water compared with -2.48 ± 0.99 in the DBs group over the 24 weeks (P=0.003) (**Table 5.2**).

5.3.5 Food intake measurement

At baseline, there was no significant difference in energy intake. Estimated energy intake measurements showed a significant reduction over time in both groups (P < 0.001 for time effect). Estimated total energy intake indicated a reduction of 417 ± 141Kcal/ d in the Water group, while this index declined 331 ± 114 Kcal/d in the DBs group over the experiment. As shown in **Table 5.3**, there was a significant group*time interaction for total energy intake over 24 wk (P = 0.005).

In addition, macronutrient intake measurements showed no significant differences between the 2 groups at baseline. However, there was a greater carbohydrate deficit in the water group than in the DB group during the 24 wk of intervention (group *time interaction, P < 0.001, **Table 5.3**)

5.4 Discussion

The purpose of the present study was to compare the effects of DBs and water consumption after lunch, as a main meal, on weight loss and also characteristics of carbohydrate and lipid metabolism in overweight and obese women with Type 2 diabetes attending a weight loss program for 24 weeks. The results of the current study showed that drinking water may lead to more weight loss, a greater improvement in fasting plasma glucose, insulin sensitivity, measured by HOMA IR and 2hpp glucose levels compared with consumption of DBs in women with Type 2 diabetes.

To my knowledge, this study was the first randomized controlled trial in women with Type 2 diabetes which has assessed the impact of excluding DBs consumption on weight loss during a voluntary integrated weight reduction program, for 24 weeks.

Weight gain and obesity are strongly related to the increased risk of type 2 diabetes while moderate weight loss improves glycaemic control (226). All of the subjects undertaking the weight loss plan had a significant weight loss. This would have been predicted given the characteristics of the prescribed treatment plan which included energy restriction, physical activity instruction and monitoring and regular patient visit and consultation in the clinic. In other rigorous clinic-based behavioral lifestyle adjustment programs, 5–10% weight losses have been reported at 6 months (216-218) which is similar to the weight losses reported in the present study. It has been shown that these comprehensive methods are more constantly effective in comparison with others recommending small but theoretically sustainable lifestyle changes that can be made to improve health (219). Furthermore, the present study showed a major reduction in waist circumference and significant improvements in cardio metabolic risk characteristics (i.e. carbohydrate and lipid metabolism characteristics) in both groups over 24 weeks, as would be predictable given the weight loss observed. Although the results indicated a significant effect of replacing DBs with water on weight loss during 24 weeks, it seems that the 24 weeks of intervention was not enough to reveal any significant effects on waist circumference (WC) as a related metabolic variable. Further longer term studies measuring metabolic effects, including WC, and more accurate assessment of body fat change using DEXA Scanning would be required.

Previous intervention studies have attempted to investigate the effects of water and DBs consumption on weight loss with inconsistent results. In a recent study, the effects of either water or DBs consumption in comparison to SSBs, without any hypoenergetic diet, and only having group behavioural counselling to promote adherence to beverage substitution were compared (127). The authors failed to find any significant differences in weight loss between water and DBs. In another recent study (128), drinking water and diet beverage was compared in subjects undergoing cognitive behavior therapy only, with no specific dietary restrictions. The result of this study showed a greater impact on weight loss with DBs compared with water. On the other hand, in a study by Dennis et al. (96), subjects who were randomly assigned to drink pre-meal water lost about 2 kg more weight than subjects on a hypoenergetic diet alone. It should be noted that the protocol of the last study (96) was not similar to the present study in that subjects in both groups had either water or DBs after their meal rather than before the meal, which is more representative of normal behaviour in this group. Furthermore, none of these studies involved obese or overweight subjects with Type 2 diabetes. Following the study reported in chapter 3 (220) indicating the beneficial effects of replacing diet beverages with water on weight loss and insulin sensitivity of obese and overweight adults, the present goal was to investigate whether these effects may be seen in women with Type 2 diabetes.

In the current study, participants drinking water after their lunch over 24 weeks lost 1.16 kg more than those in the DBs group, which is in agreement with the results in chapter 3 (220) where the overweight/ obese but otherwise healthy women in the water group lost 1.2 kg more than the DBs group. These results are also consistent with the outcome of Dennis's study (96), although their participants consumed the beverages before the meal. In contrast, present results are inconsistent with other studies which indicated either no significant change in effects on weight loss between water and DBs (127) or reported greater impact on weight loss with DBs compared with water (128).

Nevertheless, it should be mentioned that these studies had different experimental designs, for example not including any weight loss plan (127) or have cognitive behavioral therapy alone for weight loss during a shorter period of 12 weeks (128). Also, the volume of beverage, the time of drinking and the type of participants were different in these studies.

The results of this study may have arisen because the effect of replacement of DBs with water may lead to better adherence to the weight loss diet in the Water group. It has been hypothesized that artificial sweeteners may raise the hedonic desire for sweetened and more energy dense foods (115, 116, 227). Also in the current study, the effect of replacement of DBs with water on weight loss reflected better adherence to the weight-loss diet in the water group. The greater reduction in energy intake in water group compared with DBs group would have resulted in more weight loss in this group than DBs group. Moreover, more reduction of carbohydrate consumption in the water group than in the DBs group might support greater weight loss in the water group. However, in order to elucidate the mechanism that might explain the better weight loss in the Water group compared with the DBs group additional, longer term studies are required.

Like the study on healthy overweight/obese women presented in chapter 3 (220), the present study in women with Type 2 diabetes revealed a better improvement in fasting insulin sensitivity, (HOMA IR), in the Water group over the 24 weeks. There was also a beneficial impact on fasting glucose in the Water group, although the previous study in women without diabetes (220) did not show any effects on these carbohydrate metabolism characteristics. But these outcomes seen on diabetic patients were consistent with the results of the recent epidemiological study indicating daily diet beverage consumption was associated with impaired glucose control (220).

These results may have clinical implications, showing that if overweight/ obese people with Type 2 diabetes use a weight loss plan, they may have better improvements in glycemic characteristics and weight loss if they drink water and not DBs. These findings would reinforce the recommendations given in popular weight loss programs that the obese and overweight patients who are keen to lose weight should increase their water intake (94, 95). On the other hand, most obese people consider that they can drink diet beverages during a low-energy diet without any harmful effects on their weight management, and whilst they do still lose weight, the magnitude of the weight loss may be greater if they avoid DBs completely. Whilst the present study is consistent with the current guideline for increasing water consumption for better diabetes control, the results do not entirely support the recommendations indicating no deleterious effects of diet beverage on diabetes control. Since the consumption of diet soda is higher among people with diabetes than those without (228), the potential implications of studies such as the present one needs further investigation. Furthermore, as complying with replacing DBs with water for the long term may be difficult, so future research to examine the longer-term effects of enforcing Type 2 diabetes patients to replace DBs water are required.

The main strength of this study is that it was a randomized, outpatient clinical trial, whilst participants were selected from participants who wished to lose weight and control their blood sugar and included middle-aged overweight and obese women who were able to comply with weight-loss plan; hence, they demonstrated that they were motivated to adhere to the weight-loss diet protocol (225). Thirdly, subjects who participated in this study did not pay clinic fees and were provided the diet beverages for DBs group and water for the Water group which were incentives for regular by-weekly visits with the dietitian when compliance could be encouraged in both groups.

On the other hand, there are some limitations. First of all, even though the sample size provided sufficient power to distinguish statistically significant effects in the key outcome variables, the sample was not representative of the general population, mainly as it did not include men. In addition, due to the possible effects of the time of the beverage consumption, we only asked the participants to drink either water or diet beverages after the lunch in order to cover this confounding factor. Also we did not record the fluid intake of participants as it may influence satiety. Moreover the energy expenditure was not verified which would affect weight loss. Lastly, although our weekly follow up by phone call and fortnight clinic visit to measure dietary compliance of the subjects, the present study only relied upon subjective report of storing and consuming the water and DBs which is not as accurate as objective methods for measuring their compliance.

In conclusion, replacing DBs with water consumption would appear to impact beneficially on weight loss, BMI, FPG and insulin sensitivity in overweight and obese women with Type 2 diabetes following a weight loss diet. However, longer term studies are essential to see what would happen in long term in such patients.

Table 5.1 Baseline characteristics before the intervention[1]

	water group(n=41)	DBs Group(n=40)
Age(y)	34.15 (6.99)	35.45 (7.45)
Body weight(kg)	83.92 (4.42)	84.70 (7.43)
Height(cm)	159.83 (2.83)	159.65 (3.08)
BMI(kg/m²)	32.86 (1.67)	33.19 (2.25)
WC (cm)	103 (38)	102 (7)
Married	78%	82%
TC, mmol/l	4.78 (0.43)	4.75 (0.37)
HDL-C, mmol/l	1.13 (0.19)	1.13 (0.17)
LDL-C, mmol/l	2.73 (0.51)	2.71 (0.38)
TG, mmol/l	2.02 (0.27)	1.97 (0.25)
FPG, mmol/l	8.49 (0.90)	8.48 (1.03)
2hppG, mmol/l	8.82 (1.14)	8.76 (1.22)
HbA1c,%	6.97 (0.77)	6.95 (0.20)
Insulin, m U/l	19.99 (4.07)	19.84 (4.07)
HOMA-IR	7.59(1.93)	7.50 (1.89)

[1] Group difference, $P > 0.05$.

Data are presented as mean ± SD

Diet beverages: DBs, Waist circumference: WC, Total cholesterol: TC, Triglyceride: TG, Fasting plasma Glucose: FPG, 2 hour post prandial glucose: Glycated haemoglobin: HA1C,

Homeostasis model assessment of insulin resistance: HOMA-IR

Table 5.2 Anthropometric and blood measurement characteristics in Water and DBs Groups at baselien,12 and 24-week interventions*

	Water Group(n=41)			DBs Group(n=40)			P for time × group†
	Baseline	**week 12**	**week 24**	**Baseline**	**week 12**	**week 24**	
Weight, kg	83.92 (4.42)	79.96 (4.86)	77.52 (4.95)	84.70 (7.43)	81.25 (7.03)	79.45(6.99)	0.006
BMI, kg/m²	32.86 (1.67)	31.32 (2)	30.36(2.06)	33.19 (2.25)	31.84(2.13)	31.14(2.12)	0.006
WC, cm ‡	103 (5)	99 (7)	97(7)	103 (7)	99 (7)	97(6)	0.832
TC, mmol/l ‡	4.78 (0.43)	4.52 (0.45)	4.29(0.41)	4.75 (0.37)	4.49 (0.37)	4.31 (0.33)	0.119
HDL-C, mmol/l ‡	1.13 (0.19)	1.23 (0.18)	1.33(0.17)	1.13 (0.17)	1.25 (0.18)	1.33(0.16)	0.319
LDL-C, mmol/l ‡	2.73 (0.51)	2.49 (0.49)	2.22(0.46)	2.71 (0.38)	2.44 (0.40)	2.24(0.35)	0.07
TG, mmol/l ‡	2.02 (0.27)	1.77 (0.28)	1.63(0.27)	1.97 (0.25)	1.75 (0.24)	1.62(0.19)	0.639
FPG, mmol/l	8.49 (0.90)	7.76 (0.82)	6.86(0.77)	8.48 (1.03)	7.85 (0.96)	7.19(0.81)	0.005
2hpp, mmol/l	8.82 (1.14)	7.91 (0.87)	7.15(0.70)	8.76 (1.22)	8.03 (1.06)	7.40(1.01)	0.027
HbA1c,% ‡	6.97 (0.77)	6.16 (0.99)	5.80(0.82)	6.95 (0.20)	6.81 (0.17)	6.53(0.16)	0.149
Insulin, m U/l	19.99 (4.07)	16.75 (4.03)	14.27(3.81)	19.84 (4.07)	17.36 (3.43)	17.36(3.43)	0.011
HOMA-IR	7.59(1.93)	5.80(1.62)	4.39 (1.37)	7.50 (1.89)	6.05 (1.39)	5.01(1.20)	0.003

* Data are presented as mean (SD) for the 81 participants

† P values are for Water relative to DBs group (time × Group interaction) by a linear mixed model analysis with repeated measures.

‡ Significant main effect of time, P < 0.001

Diet beverages: DBs, Waist circumference :WC, Total cholesterol: TC, Triglyceride: TG, Fasting plasma glucose: FPG
Glycated hemoglobin: HbA1c, 2 hour post prandial: 2hpp,Homeostasis model assessment of insulin resistance: HOMA-IR

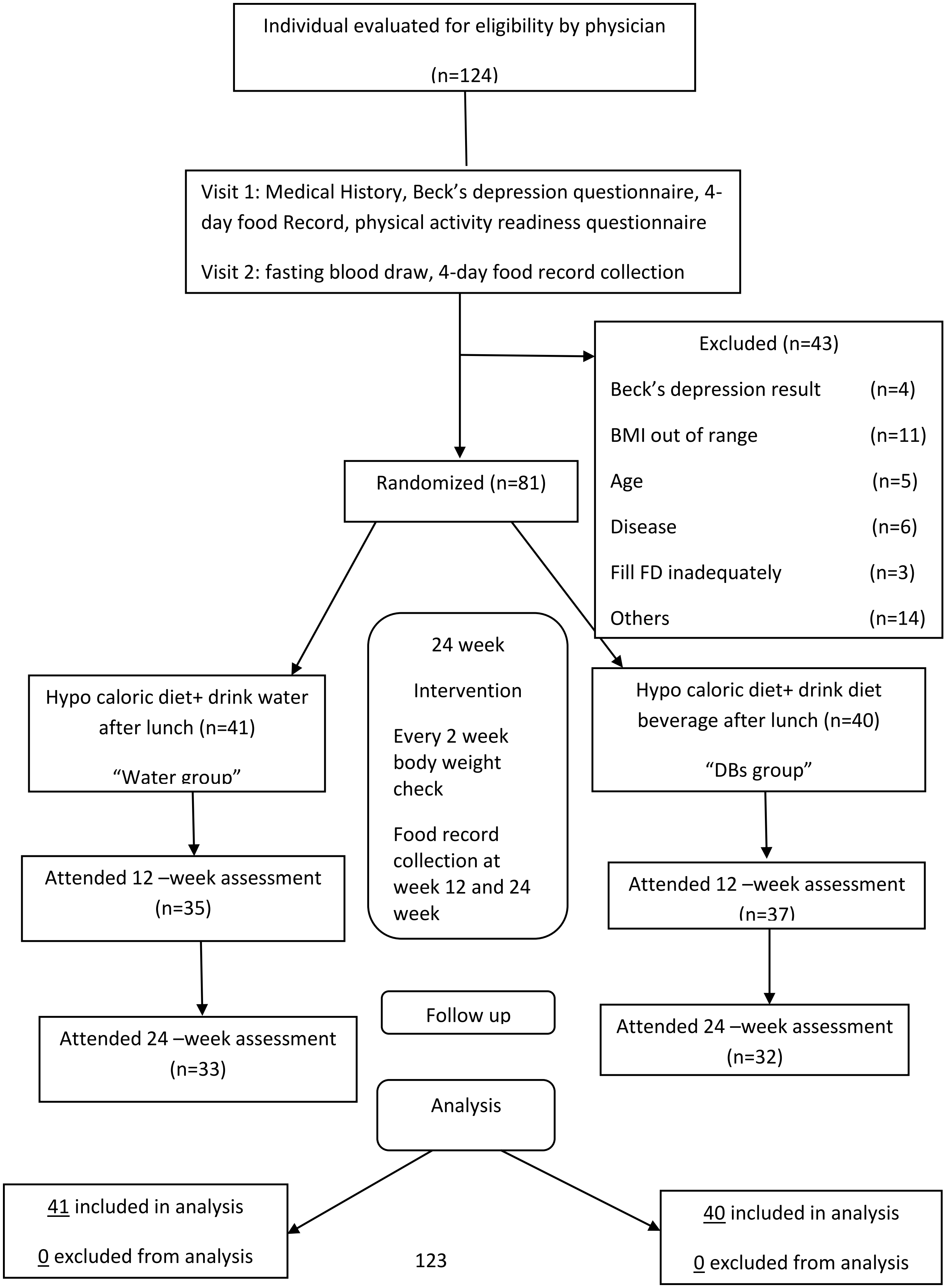
Table 5.3 Self- reported dietary intake in Water and DBs Groups before and after the 24-week interventions[1]

Intake	Water Group(n=41)		DBs Group(n=40)		P for time × group[2]
	Baseline	week 24	Baseline	week 24	
Total Energy (kcal)	2202(173)	1785(146)	2157(275)	1827(302)	0.005
Protein (g)	81.2(8.7)	79.6(9.4)	80.4(13.9)	80(15.6)	0.240
Protein (%)	14.8%(2)	17.9%(1.6)	14.9%(2.1)	17.5%(1.8)	
Fat (g)	86.2(11.7)	63.8(8.1)	82.6(16.6)	61.1(38)	0.675
Fat (%)	35.1%(2.8)	32.1%(2.3)	34.4%(4.3)	30.1%(2.4)	
Carbohydrate (g)	275.4(20.9)	223(17.6)	273.1(34.5)	239.1(38.6)	<0.001
Carbohydrate (%)	50.1%(1.7)	50%(2.5)	50.7%(3.2)	52.4%(2.4)	
Fibre (g)	20.7(5.3)	22.2(5.3)	20.8(3.1)	22.2(3.2)	0.280

[1] Data are presented as mean ± SD for the 81 participants

[2] P values are for Water relative to DBs group (time × Group interaction) by repeated-measures two way ANOVA

Figure 5.1 Screening, enrollment, randomization and follow up of study participants



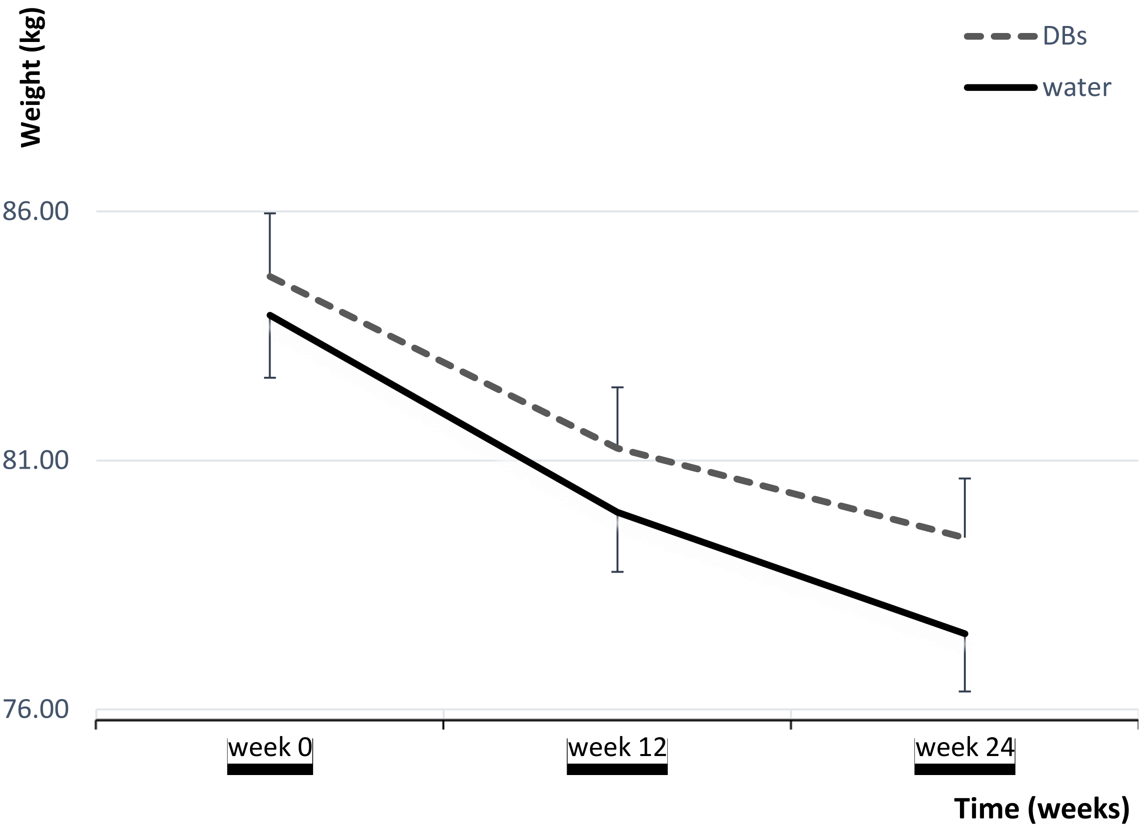


Figure 5.2 Mean (SE) weight at baseline, 12 and 24 wk of energy restriction with either drinking water (Water; n = 41) or diet beverages (DBs; n = 40) in all participants, regardless of attrition. $P < 0.001$ for the main effect of time.

There was also a significant difference in weight reduction between the two groups after 24 weeks (P=0.006), based on linear mixed effects models.

Chapter 6.General discussion

The aim of this thesis was to evaluate the effects of replacing diet beverages with water on weight loss and cardiovascular risk factors in healthy and also Type 2 diabetic obese and overweight women during a hypoenergetic diet.

The prevalence of obesity and diabetes has increased over the last decades (229) which is in parallel with an increase in the total energy intake. One of the factors which may enhance energy overconsumption is calorically sweetened beverages. For example, the overconsumption of high-fructose corn syrup in such beverages may play a role in the epidemic of obesity (72). In fact, the amount of energy consumed from beverages has increased and provides a significant source of daily energy intake in adults (81). In addition, a higher intake of sugar sweetened beverages (SSBs) is related with the development of metabolic syndrome and an increased risk of Type 2 diabetes (80).

On the other hand, replacing sugar with low- or zero-calorie sweeteners is a common approach for helping weight control which offers sweet taste with less or no energy and helps to lower the energy density of beverages and some foods (192, 198, 197). Actually, reduced dietary energy density should result in lower energy intakes but there are arguments that intense sweeteners increase the appetite for sweet foods, stimulate overeating and may even lead to weight gain (197).

Nutritionists and health professionals generally advise the general public who wish to lose weight to raise their water consumption, a tactic often recommended in popular weight loss programs (94, 95). In fact, water is also the recommended beverage for maintaining good health (230).

To my knowledge, no intervention studies have assessed the impact of replacing DBs consumption with water on weight loss of healthy and Type 2 diabetic obese and overweight patient during a voluntary integrated weight reduction program. However, like other nutritional interventions, there is concern that any results observed in the short-term may not exist over a longer time period, especially due to a maintained desire for sweet tasting food and drinks in many obese people. Hence, the purpose of this thesis was to evaluate:

1. Effects of replacing diet beverages with water during a hypoenergetic diet on obese and overweight women during a 24 weeks clinical trial.
2. Effects of replacing diet beverages with water during a hypoenergetic diet on type 2 diabetic obese and overweight women during a 24 weeks clinical trial.
3. Effects of replacing diet beverages with water on weight loss and weight maintenance: 18 month follow up, randomized clinical trial

6.1 Strengths, Limitation and future studies

In this thesis, substantial attempts were made to overcome the limitation of previous work on the effect of DBs on weight and health. The main strength of this thesis is that the studies were randomized, outpatient clinical trials, whilst participants were on a comprehensive diet plan for weight control in a long term intervention, whereas in previous studies which compared effects of water and DBs on weight changes, there was no such comprehensive diet plan (127, 128). In addition, all participants of the studies in this thesis wished to lose weight and included middle-aged overweight and obese women who were able to comply with a weight loss plan, and were motivated to adhere to the weight loss diet protocol (225). Also, providing the diet beverages for DBs group and water for the Water group and also providing the comprehensive diet plan free to the participants were incentives for them to come to the clinic for regular bi-weekly visits and compliance was good in both groups.

However, it should be admitted that this thesis includes some limitations. The studies were all on women, which was done to avoid possible effects of gender. Furthermore, experience has shown that women tend to be more motivated and work harder to lose weight and attend regular visits, and are more compliant than men in dietary interventions (225).

Despite the noticeable attempts to control the confounding factors in the free-living state, it is likely that these studies could not control all of them which may have created some errors in the interpretation and generality of the results.

There may also be some inaccuracy in extrapolating the energy intake record as previous studies indicate under –reporting of energy intake (231, 232) which can introduce some errors in the interpretation of outcomes. The studies presented in this thesis may have been affected by under-reporting, especially in obese participants.

Moreover, even though the sample size providing sufficient power to distinguish statistically significant effects in the key outcome variables, the sample was not representative of the general population, mainly as it did not include men.

Furthermore, the fluid intake of participants in all studies was not recorded, while it may influence satiety (233). In addition, the energy expenditure, as a key component of energy balance, was not verified which would affect weight loss. Lastly, although the participants had a weekly follow up by phone call and fortnightly clinic visit to measure dietary compliance of the participants but the present studies , as free life studies, only relied upon subjective report of storing and consuming the water and DBs which is not as accurate as objective methods for measuring their compliance.

Overall, longer-term studies involving both genders (and even the same type of study on children and adolescents) are required to investigate whether the beneficial effects of replacing diet beverages with water during a hypoenergetic diet on human metabolism, the intake of sweet foods and weight loss are sustained.

6.2 Effects on body weight, BMI and waist circumference

Significant differences were found in body weight and BMI changes between groups of healthy overweight and obese women over 24 weeks and also at the 18 month (77 weeks) follow up. Similarly, in Type 2 diabetic obese and overweight women, significant changes were seen in body weight and BMI between groups during the 24 weeks intervention.

No significant differences were seen between the groups in the reduction of waist circumference over the studies.

These results are consistent with Stookey et al who reported greater weight loss in water consumers on a hypoenergetic diet compared with those who consumed sweetened beverages (202). Furthermore in the study of Dennis et al (96), participants who drank pre-meal water lost more weight than participants on the hypoenergetic diet alone. These studies showed significant effects of type of beverage consumption and weight loss, suggesting beneficial effects of drinking water.

In contrast, the present results are inconsistent to some other studies. The two recent studies either showed no significant differences, in terms of weight loss, between drinking water and DBs (127) or even indicated greater impact on weight loss with DBs compared with water (128). However, it should be noted that these prior studies had differences in the protocol such as not including any weight loss plan (127) or have only cognitive behavioral therapy for weight loss (128). Additionally, in previous studies, both with confirming and opposing results, the volume of beverage and the time of drinking water and beverage were different.

It should be also added that the results of many epidemiological studies are in agreement with the results of my studies. For example Fowler and colleagues observed a classic, positive dose-response relationship between artificial sweetener beverage consumption and long-term weight gain (200). The results of Stellman et al furthermore showed that users of artificial sweetener were significantly more likely than nonusers to gain weight, regardless of their initial weight (221).

The beneficial effect of replacement of DBs with water on weight loss in these studies may reflect better adherence to the multidisciplinary weight loss plan in the water group leading to a greater reduction in energy intake, which is consistent with previous findings (198). One possibility which could explain more weight loss with water consumption compared with DBs may be that people know that water is a "health" drink, and if they are in a protocol trying one healthy option, it might be easier to do more healthy things. Also, the greater reduction in total carbohydrate intake seen in the water group compared to the DB group would also contribute to the greater weight loss in the water group. This finding in these studies is also consistent with the suggestion that the use of non- nutrient sweetener within DBs may be associated with the sustained consumption of sweet foods or high-energy foods compared to drinking water (200).

However, further studies should consider whether the amount of water, the timing or other factors could impact on the effects of replacement of diet beverage with water, on weight loss and anthropometric measurements.

6.3 Effects on Glucose metabolism

Significant between groups differences were observed for fasting insulin, 2hpp plasma glucose and HOMA-IR among water and DBs groups over 24 weeks in healthy and Type 2 diabetic obese and overweight women. Furthermore, these beneficial effects on carbohydrate metabolism were sustained over 18 month follow up in healthy overweight and obese women. There was also a significant difference in the changes between the groups for HbA_{1C} after 18 month follow up although HbA_{1C} was not significantly different between treatments after 24 weeks in healthy and Type 2 diabetic obese and overweight women.

Furthermore, between group differences were significant for fasting plasma glucose in Type 2 diabetic obese and overweight women after 24 weeks intervention although there were no significant changes among groups for FPG in healthy obese and overweight women after 24 weeks and after 77 weeks follow up.

Similar to the study on healthy overweight/obese women, the study on women with Type 2 diabetes showed a better improvement in fasting insulin sensitivity, (HOMA IR), in the Water group over the 24 weeks. The better improvement of insulin sensitivity in the water group compared with DBs group remained after the 77 weeks follow up. The outcomes seen with diabetic patients were consistent with the results of the latest epidemiological study indicating daily diet beverage consumption was associated with impaired glucose control (227).

Similar to the result of the studies of this thesis, the recent study by Suez J et al (234), demonstrated that frequent consumption of non-caloric artificial sweeteners (NAS) drives the development of glucose intolerance in association with induction of compositional and functional alterations to the intestinal microbiota. They identified NAS altered microbial metabolic pathways that are linked to host susceptibility to metabolic disease, and demonstrate similar NAS-induced glucose intolerance in healthy human participants.

Some theories explaining the effect of aspartame on gut metabolism have been suggested but the exact mechanism has not been defined. One theory suggests that Phenylalanine which is one of the breakdown products of aspartame, could play a major role in regard to the impact of aspartame on metabolic disease (236).

It should be mentioned that Phenylalanine is known inhibitor of Intestinal alkaline phosphatase(IAP) and IAP is a gut enzyme important in intestinal microbial homeostasis. It has been hypothesized that the negative metabolic effect seen with aspartame consumption could be due to its indirect inhibition of IAP through the breakdown product Phenylalanine (236).

Further investigations are needed to understand the exact mechanism behind the beneficial effects of replacing diet beverage with water on carbohydrate metabolism and potential impact on intestinal microbiota.

6.4 Effects on lipid profile

Lipid profiles, including fasting total plasma cholesterol, low-density lipoprotein cholesterol levels, and triglyceride concentration, were improved in both parallel intervention groups in all of the studies in this thesis, as expected due to the weight loss interventions. The reduction of fasting total plasma cholesterol, low-density lipoprotein cholesterol levels, and triglyceride concentration were not significantly different between Water and DBs groups in the 24 weeks intervention on healthy overweight and obese women. There were also no significant differences in the increase in high density lipoprotein cholesterol between the two groups over 24 weeks.

Moreover, the follow up study showed no significant changes between the groups for TC, TG, HDL-C and LDL-C over the 77 weeks. Similar results were also observed in the overweight and obese participants with Type 2 diabetes during 24 weeks of intervention.

6.5 Effects on energy intake

The study on healthy overweight and obese and Type 2 diabetic obese and overweight women showed no significant difference in energy intake at base line. However over the time of the intervention, there were significant reductions in the recorded mean total energy intake and the macronutrient composition of the diet in both groups ($P < 0.001$ for time effect). No significant differences were observed in the macronutrient composition between the water group and DBs group at baseline. However, there was a greater carbohydrate reduction in the water group compared with the DBs group during the 24 weeks of intervention (group by time interaction, $P= 0.01$). The same results were observed at 77 weeks follow up in the healthy overweight and obese participants.

The greater reduction in energy intake and carbohydrate consumption in the water group compared with the DBs group would support greater weight loss in the water group. These results indicate that consumption of water in obese and overweight women following a diet plan may tend to better weight loss due to better reduction in food intake in comparison with DBs consumer.

There is evidence that consuming food believed to be low in calorie content can produce reduced satiety and lead to “rebound” eating [123,124], however not all studies support such an outcome

[125,126].These findings raise the possibility that NNSs might affect intake and metabolism simply by creating the impression that a food or beverage is less caloric than its actual energy content. There is also the possibility that repeated oral receptor stimulation by high affinity NNSs may enhance sensory adaptation and reduce sweet taste sensitivity, which may in turn influence the acceptability of caloric and non-caloric sweeteners with increased preference thresholds of sweet foods.

However, further studies are needed to assess sweet foods intake in relation to water or DB intake.

6.6 Clinical implication and conclusion

This thesis supports the usual health professionals' recommendation for obese people to drink only water for better weight loss. But the results are inconsistent with the belief that obese people can enjoy sugary taste by drinking diet beverages and are not required to limit themselves on only drinking water to achieve weight loss during a multidisciplinary weight loss program. It is likely that drinking water vs. drinking DBs, with lunch, as an alternative of DBs helps obese and overweight persons lose more weight. Drinking water instead of DBs also appears to have a beneficial impact on carbohydrate metabolism via improvement in insulin sensitivity.

From the clinical point of view in obesity treatment, the results would support the recommendations given in popular weight loss programs that the obese and overweight patients who are keen to lose weight should increase their water intake (94, 95). Even though most obese people consider that they can drink diet beverages during a low-energy diet without any harmful effects on their weight management, and whilst they do still lose weight, the result of my studies shows that the amount of the weight loss may be greater if they avoid DBs completely and drink water only.

The previous evidence indicated the consumption of diet soda is higher among people with diabetes than those without (228), as they assume they can drink DBs and still control their blood glucose. The study in this thesis on overweight and obese women with Type 2 diabetes revealed if they use a weight loss plan, they may have better improvements in glycemic characteristics and more weight loss if they drink water instead of diet beverages. Meanwhile the potential implications and mechanisms behind interventions such as the present one needs further investigation. Furthermore, as complying with replacing DBs with water for the long term may be difficult, so future research needs to examine the longer-term effects of requiring Type 2 diabetes patients to replace DBs with water.

Finally, high-quality, long duration controlled feeding studies with accurate measurement of food and beverage intake and types of food consumed in both genders are required. This should be done with the aim of finding the actual mechanism behind the effect of replacing diet beverages with water on weight loss and carbohydrate metabolism in adults during a hypoenergetic diet.

References

1. Strauss R, Pollack H. Epidemic increase in childhood overweight, 1986-1998. JAMA. 2001;286(22):2845-8.

2. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of childhood and adult obesity in the united states, 2011-2012. JAMA. 2014;311(8):806-14.

3. Haslam D, James W. Obesity, Lancet. 2005;366:1197–209.

4. Manson J, Colditz G, Stampfer M, Willet W, Rosner B, Monson R, et al. A prospective study of obesity and risk of coronary heart disease in women. N Engl J Med. 1990:882-9.

5. Wilson P, D'Agostino R, Sullivan L, Parise H, Kannel W. Overweight and obesity as determinants of cardiovascular risk: the Framingham experience. Arch Intern Med. 2002;162:1867-72.

6. Kotsis V, Stabouli S, Papakatsika S, Rizos Z, Parati G. Mechanisms of obesity-induced hypertension. Hypertens Res. 2010;33(5):386-93.

7. Aneja A, El-Atat F, McFarlane S, Sowers J. Hypertension and obesity. Recent Prog Horm Res. 2004;59:169-205.

8. Cecchini M, Sassi F, Lauer J, Lee Y, Guajardo-Barron V, Chisholm D. Tackling of unhealthy diets, physical inactivity, and obesity: health effects and cost-effectiveness. The Lancet. 2010;376(9754):1775-84.

9. Popkin B, Gordon-Larsen P. The nutrition transition: worldwide obesity dynamics and their determinants. International journal of obesity. 2004;28:S2-S9.

10. National Institutes of Health. National Heart, Lung, and Blood Institute. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults: The evidence report. Obes Res. 1998;6 Suppl 2:515.

11. World Health Organization (WHO). “WHO BMI Classification". Global Database on Body Mass Index. 2006.

12. Report of a WHO Consultation on Obesity: Preventing and managing the global epidemic. Geneva, 3-5 June 1997. World Health Organization, Geneva. 1998.

13. Flodmark C, Lissau I, Moreno L. New insights into the field of children and adolescents' obesity : the European perspective. Int J Obes Relat Metab Disord 2004;28:1189.

14. Deurenberg P, Weststrate J, Seidell J. Body mass index as a measure of body fatness: age- and sex-specific prediction formulas. Br J Nutr 1991(65):105.

15. Boudewijn K, Jan Willem F, Manuel C. Dyslipidemia in Obesity: Mechanisms and Potential Targets. Nutrients. 2013;5:1218–40.

16. Scott K, Bruffaerts R, Simon G, Alonso J, Angermeyer M, de Girolamo G, et al. Obesity and mental disorders in the general population: results from the world mental health surveys. International Journal of Obesity. 2007;32(1):192-200.

17. Wolin K, Carson K, Colditz G. Obesity and cancer. The oncologist. 2010;15(6):556-65.

18. Chan J, Rimm E, Colditz G, Stampfer M, Willett WC. Obesity, fat distribution , and weight gain as risk factors for clinical diabetes in men. Diabetes Care 1994;17:961-9.

19. Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. The Lancet. 2004;364(9438):937-52.

20. Razay G, Vreugdenhil A, Wilcock G. Obesity, Abdominal Obesity and Alzheimer Disease. Dementia and Geriatric Cognitive Disorders. 2006;22(2):173-6.

21. Anjana M, Sandeep S, Deepa R, Vimaleswaran K, Farooq S, Mohan V. Visceral and Central Abdominal Fat and Anthropometry in Relation to Diabetes in Asian Indians. Diabetes Care. 2004;27(12):2948-53.

22. Kopelman PG, Caterson ID, Dietz WH. Clinical obesity in adults and children: John Wiley & Sons; 2009.

23. Finucane MM, Stevens GA, Cowan MJ, Danaei G, Lin JK, Paciorek CJ, et al. National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9· 1 million participants. The Lancet. 2011;377(9765):557-67.

24. Vasan R, Pencina M, Cobain M. Estimated risks for developing obesity in the Framingham Heart Study. Ann Intern Med. 2005;143:143:473.

25. Seidell J. Time trend in obesity: an epidemiological perspective. Horm Metab Res. 1997;29:155-8.

26. Berghofer A, Pischon T, Reinhold T, Apovian C, Sharma A, Willich S. Obesity prevalence from a European perspective: a systematic review. BMC Public Health. 2008;8(1):200.

27. Flegal M, M. C, Kuczmarski R. Overweight and obesity in the United States: prevalence and trends, 1960-1994. Int J Obes Relat Metab Disord. 1998;22(22):39-48.

28. Rashidy-Pour A, Malek M, Eskandarian R, Ghorbani R. Obesity in the Iranian population. Obes Rev. 2009;10:2–6.

29. Esteghamati A, Khalilzadeh O, Mohammad K, Meysamie A, Rashidi A, Kamgar M, et al. Secular trends of obesity in Iran between 1999 and 2007: National Surveys of Risk Factors of Non-communicable Diseases. Metabolic syndrome and related disorders. 2010;8(3):209-13.

30. Ayatollahi S, Ghoreshizadeh Z. Prevalence of obesity and overweight among adults in Iran. Obesity Reviews. 2010;11(5):335-7.

31. Lau D, Douketis J, Morrison K, Hramiak I, Sharma A. 2006 Canadian clinical practice guidelines on the management and prevention of obesity in adults and children summary. CMAJ 2007;176(8):S1–13.

32. Bleich S, Cutler D, Murray C, Adams A. Why is the developed world obese? Annu Rev Public Health 2008;29:273–95.

33. Mokdad A, Marks J, Stroup D, Gerberding J. Correction: actual causes of death in the United States. JAMA. 2005:293:.

34. Peeters A, Barendregt J, Willekens F, Mackenbach J, Al Mamun A, Bonneux L. Obesity in adulthood and its consequences for life expectancy: A life-table analysis. Annals of Internal Medicine. 2003;138(1):24–32.

35. Keith SW, Redden DT, Katzmarzyk PT, Boggiano MM, Hanlon EC, Benca RM, et al. Putative contributors to the secular increase in

obesity: exploring the roads less traveled. Int J Obes. 2006;30(11):1585-94.

36. Flegal KM, Graubard B, Williamson D, Gail M. Excess deaths associated with underweight, overweight, and obesity. JAMA. 2005:293:1861.

37. Drewnowski A, Specter S. Poverty and obesity: the role of energy density and energy costs. Am J Clin Nutr. 2004;79(1):6–16.

38. James W. The fundamental drivers of the obesity epidemic. Obes Rev 2008:6–13.

39. Nestle M, Jacobson M. Halting the obesity epidemic: a public health policy approach. Public Health Rep. 2000;115(1):12–24.

40. Kushner R. Treatment of the Obese Patient(Contemporary Endocrinology). Totowa, NJ: Humana Press; 2007 Retrieved April 5, 2009.

41. Langlois K, Garriguet D, Findlay L. Diet composition and obesity among Canadian adults. Health Rep. 2009;20(4):11-20.

42. American Academy of Pediatrics, Committee on School Health. Soft drinks in schools. Pediatrics. 2004;113:152-4.

43. Flega lK, Carroll M, Ogden C, Johnson C. Prevalence and trends in obesity among US adults, 1999–2000. JAMA. 2002;288(14):1723–7.

44. Wright J, Kennedy-Stephenson J, Wang C, McDowell M, CL. J. trends in intake of energy and macronutrients—United States, 1971–2000. Morb Mortal Wkly Rep. 2004;53(4):80–2.

45. Caballero B. The global epidemic of obesity: An overview. Epidemiol Rev. 2007;29:1–5.

46. Mozaffarian D, Hao T, Rimm E, Willett W, Hu F. Changes in Diet and Lifestyle and Long-Term Weight Gain in Women and Men. The New England Journal of Medicine. 2011;364(25):2392–404.

47. Malik VS SM, Hu FB Intake of sugar-sweetened beverages and weight gain: a systematic review. Am J Clin Nutr. 2006;84 (2): 274–88.(2):274–88.

48. Olsen N, Heitmann B. Intake of calorically sweetened beverages and obesity. Obes Rev 10 2009;10(1):68–75.

49. Rezazadeh A, Rashidkhani B. The Association of General and Central Obesity with Major Dietary Patterns in Adult Women Living in Tehran, Iran. ARYA Atherosclerosis. 2010;6(1):23-30.

50. Azadbakht L, Esmaillzadeh A. Dietary and non-dietary determinants of central adiposity among Tehrani women. Public Health Nutrition. 2008;11(05):528-34.

51. Malekzadeh R, Mohamadnejad M, Merat S, Pourshams A, Etemadi A. Obesity pandemic: an Iranian perspective. Arch Iranian Med. 2005;8(1):1-7.

52. Yan L, Daviglus M, Liu K. Midlife body mass index and hospitalization and mortality in older age. JAMA. 2006:295:190.

53. legal K, Graubard B, Williamson D, Gail M. Cause-specific excess deaths associated with underweight, overweight, and obesity. JAMA. 2007:298:2028.

54. McTigue K, Larson J, Valoski A. Mortality and cardiac and vascular outcomes in extremely obese women. JAMA. 2006:296:79.

55. Whitlock G, Lewington S, Sherliker P. Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. The Lancet 2009;373:1083–96.

56. Sweeting H. Measurement and Definitions of Obesity In Childhood and Adolescence: A field guide for the uninitiated. Nutr J. 2007;6:32.

57. Doll H, Petersen S, Stewart-Brown S. Obesity and physical and emotional well-being: associations between body mass index, chronic illness, and the physical and mental components of the SF-36 questionnaire. Obesity research. 2000;8(2):160-70.

58. Field A, Coakley E, Must A, Spadano J, Laird N, Dietz W, et al. Impact of overweight on the risk of developing common chronic diseases during a 10-year period. Archives of Internal Medicine. 2001;161(13):1581-6.

59. Shoelson S, Lee J, Goldfine A. Inflammation and insulin resistance. J Clin Invest. 2006;116:1793–801.

60. Dentali F, Squizzato A, Ageno W. The metabolic syndrome as a risk factor for venous and arterial thrombosis. Semin Thromb Hemost. 2009;35(5):451–7.

61. Westphal S. Obesity, abdominal obesity, and insulin resistance. Clin Cornerstone. 2008;9(1):23-9.

62. Morkedal B, Romundstad P, Vatten L. informativeness of indices of blood pressure, obesity and serum lipids in relation to ischaemic heart disease mortality: the HUNT-II study. European Journal of Epidemiology 2011;26(6):457–61.

63. Nguyen NT MC, Lane KT. Association of hypertension, diabetes, dyslipidemia, and metabolic syndrome with obesity: findings from the National Health and Nutrition Examination Survey,1999 to 2004. J Am Coll Surg 2008:207:928.

64. Campfield L, Smith F. Blood glucose dynamics and control of meal initiation: a pattern detection and recognition theory. Physiological Reviews. 2003;83(1):25-58.

65. US Department of Health and Human Services. US Department of Agriculture. Dietary Guidelines for Americans. 2005.

66. Woodward-Lopez G, Kao J, Ritchie L. To what extent have sweetened beverages contributed to the obesity epidemic? . Public Health Nutrition 2010;14(3):499-509.

67. Malik V, Popkin B, Bray G, Despres J, Hu F. Sugar-Sweetened Beverages, Obesity, Type 2 Diabetes Mellitus, and Cardiovascular Disease Risk. Circulation. 2010;121(11):1356-64.

68. Hu F, Malik V. Sugar-sweetened beverages and risk of obesity and type II diabetes: epidemiologic evidence. Physiology and Behavior. 2010;100: 47-54.

69. Malik V, Schulze M, Hu F. Intake of sugar-sweetened beverages and weight gain: a systematic review. The American journal of clinical nutrition. 2006;84(2):274-88.

70. Malik V, Pan A, Willett W, Hu F. Sugar-sweetened beverages and weight gain in children and adults: a systematic review and meta-analysis. The American journal of clinical nutrition. 2013;98(4):1084-102.

71. Wang Y, Ludwig D, Sonneville K, Gortmaker S. Impact of change in sweetened caloric beverage consumption on energy intake among children and adolescents. Arch Pediatr Adolesc Med. 2009;163(4):336-43.

72. Bray G, Nielsen S, Popkin B. Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. The American journal of clinical nutrition. 2004;79(4):537-43.

73. Tordoff M, Alleva A. Effect of drinking soda sweetened with aspartame or high-fructose corn syrup on food intake and body weight. Am J Clin Nutr. 1990;51:963–9.

74. Tappy L, Le K. Metabolic effects of fructose and the worldwide increase in obesity. Physiological reviews 2010;90(1):23–46.

75. Lyssiotis C, Cantley L. Metabolic syndrome: F stands for fructose and fat. Nature. 2013;502(7):181–2.

76. Vartanian L, Schwartz M, Brownell K. Effects of Soft Drink Consumption on Nutrition and Health: A Systematic Review and Meta-Analysis. American Journal of Public Health. 2007;97(4):667-75.

77. Avena N, Rada P, Hoebel B. Evidence for sugar addiction: behavioral and neurochemical effects of intermittent, excessive sugar intake. Neuroscience & Biobehavioral Reviews. 2008;32(1):20-39.

78. Assy N, Nasser G, Kamayse I, Nseir W, Beniashvili Z, Djibre A, et al. Soft drink consumption linked with fatty liver in the absence of traditional risk factors Canadian Journal of Gastroenterology. 2008;22(10):811-6.

79. Dhingra R, Sullivan L, Jacques P, Wang T, Fox C, Meigs J, et al. soft drink consumption and risk of developing cardiometabolic risk factors and the metabolic syndrome in middle-aged adults in the community. Circulation. 2007;116(5):480-8.

80. Malik V, Popkin B, Bray G, Despres J, Willett W, Hu F. Sugar-sweetened beverages and risk of metabolic syndrome and type 2 diabetes: a meta-analysis. Diabetes Care 2010;33:2477–83.

81. Duffey K, Popkin B. Shifts in patterns and consumption of beverages between 1965 and 2002 Obesity 2007;15:2739–47.

82. Chen L, Appel L, Loria C, Lin P, Champagne C, Elmer P, et al. Reduction in consumption of sugar-sweetened beverages is associated with weight loss: the PREMIER trial. Am J Clin Nutr. 2009;89:1299–306.

83. Dennis E, Flack K, Davy B. Beverage consumption and adult weight management: a review Eat Behavior. 2009;10:237–46.

84. DellaValle D, Roe L, Rolls B. Does the consumption of caloric and non-caloric beverages with a meal affect energy intake? Appetite. 2005;44(2):187-93.

85. Flood J, Roe L, Rolls B. The effect of increased beverage portion size on energy intake at a meal. J Am Diet Assoc. 2006;106:1984–90.

86. Ogden C, Carroll M, Kit B, Flegal K. Prevalence of obesity and trends in body mass index among US children and adolescents, 1999-2010. JAMA 2012;307:483-90.

87. Olds T, Maher C, Zumin S. Evidence that the prevalence of childhood overweight is plateauing: data from nine countries. Int J Pediatr Obes 2011;6:342-60.

88. Mattes R. Fluid calories and energy balance the good, the bad, and the uncertain. Physiol Behav 2006;89:66-70.

89. Krebs N, Jacobson M. Prevention of pediatric overweight and obesity. Pediatrics. 2003;112:424–30.

90. Ludwig D, Peterson K, Gortmaker S. Relation between consumption of sugar-sweetened drinks and childhood obesity: a prospective, observational analysis. The Lancet. 2001;357:505–8.

91. Berkey C, Rockett H, Field A, Gillman M, Coldit G. Sugar-added beverages and adolescent weight change. Obes Res. 2004;12:778–88.

92. Schulze M, Manson J, Ludwig D. Sugar-sweetened beverages, weight gain, and incidence of type 2 diabetes in young and middle-aged women. JAMA. 2004;292:927–34.

93. Welsh J, Cogswell ME, Rogers S, Rockett H, Mei Z, Grummer-Strawn L. Overweight among low-income preschool children associated with the consumption of sweet drinks: Missouri, 1999–2002. Pediatrics. 2005;115(2).

94. Sears B, Lauren W. The Zone. New York: Harper Collins; 1995.

95. Atkins R. Atkins' NEW Diet Revolution. New York: Avon Books; 1992.

96. Dennis E, Dengo A, Comber D, Flack K, Savla J, Davy K, et al. Water consumption increases weight loss during a hypocaloric diet intervention in middle-aged and older adults Obesity 2010;18:300-7.

97. Rodin,J. Comparative effects of fructose, aspartame, glucose, and water preloads on calorie and macronutrient intake. . Am J Clin Nutr 1990(51):428–35.

98. Black RM, Leiter LA, Anderson GH. Consuming aspartame with and without taste: differential effects on appetite and food intake of young adult males. Physiol Behav 1993;53:459–66.

99. Black RM, Tanaka P, Leiter LA, Anderson GH. Soft drinks with aspartame: effect on subjective hunger, food selection and food intake of young adult males. Physiol Behav. 1991;49:803–10.

100. Beridot-Therond M, Arts I, Fantino M, de la Gueronniere V. Short-term effects of the flavor of drinks on ingestive behaviors in man. Appetite 1998;31:67–81.

101. Lavin JH, French SJ, Read NW. The effect of sucrose- and aspartame-sweetened drinks on energy intake, hunger and food choice of female, slightly restrained eaters. Int J Obes Relat Metab Disord. 1997;21:37–42.

102. Porikos KP, Hesser MF, van Itallie TB. Caloric regulation in normal-weight men maintained on a palatable diet of conventional foods. Physiol Behav. 1982;29:293–300.

103. Popkin B, Barclay D, Nielsen S. Water and food consumption patterns of U.S. adults from 1999 to 2001. Obes Res. 2005;13:2146–52.

104. Stookey JD, Constant F, Gardner CD, Popkin BM. Replacing sweetened caloric beverages with drinking water is associated with lower energy intake. Obesity (Silver Spring). 2007;15:3013–22.

105. Stookey JD, Constant F, Popkin BM, Gardner CD. Drinking water is associated with weight loss in overweight dieting women independent of diet and activity. Obesity 2008;16(16):2481–8.

106. Lappalainen R, Mennen L, van Weert L, Mykkanen H. Drinking water with a meal: a simple method of coping with feelings of hunger, satiety and desire to eat. Eur J Clin Nutr. 1993;47:815–9.

107. Van Walleghen E, Gentile C, Davy B. Pre-meal water consumption reduces meal energy intake in older but not younger subjects. Obesity 2007;15:93–9.

108. Rolls BJ, Kim S, Fedoroff IC. . Effects of drinks sweetened with sucrose or aspartame on hunger, thirst and food intake in men. Physiol Behav 1990;48:19–26.

109. Canty DJ CM. Effects of consumption of caloric vs noncaloric sweet drinks on indices of hunger and food consumption in normal adults. Am J Clin Nutr 1991;53:159–1164.

110. Davy B, Dennis E, Dengo A, Wilson K, Davy K. Water consumption reduces energy intake at a breakfast meal in obese older adults. J Am Diet Assoc. 2008;108:1236–9.

111. Report of a WHO Consultation on Obesity. preventing and managing the global epidemic of Obesity Geneva: World Health Organization, 2000.

112. Foreyt J, Kleinman R, Brown R, Lindstrom R. The use of low-calorie sweeteners by children: implications for weight management. J Nutr 2012;142:1155S-62S.

113. Fowler SP,Williams K, Resendez RG, Hunt KJ, Hazuda HP, Stern MP. Fueling the obesity epidemic? Artificially sweetened beverage use and long-term weight gain. Obesity (Silver Spring) 2008;16:1894-1900. 2008;16.

114. Drewnowski A. Intense sweeteners and energy density of foods: implications for weight control. Eur J Clin Nutr 1999;53:757–63.

115. Blundell JE, Hill AJ.Paradoxical effects of an intense sweetener (aspartame) on appetite. Lancet 1986;1:1092–3.

116. Rogers P, Blundell J. Separating the actions of sweetness and calories: effects of saccharin and carbohydrates on hunger and food intake in human subjects. Physiol Behav 1989;45:1093–9.

117. Rolls BJ, Laster LJ, Summerfelt A. Hunger and food intake following consumption of low-calorie foods. Appetite 1989;13:115–27.

118. Flatt J. The difference in storage capacities for carbohydrate and for fat, and its implications in the regulation of body weight. Ann N Y Acad Sci 1987;499:104–23.

119. Astrup A, Raben A. Obesity: an inherited metabolic deficiency in the control of macronutrient balance? . Eur J Clin Nutr 1992;46:611–20.

120. Lissner L, Levitsky D, Strupp B, Kalkwarf H, Roe D. Dietary fat and the regulation of energy intake in human subjects. Am J Clin Nutr 1987;46:886–92.

121. Saris W, Astrup A, Prentice A. Randomized controlled trial of changes in dietary carbohydrate/fat ratio and simple vs complex carbohydrates on body weight and blood lipids. Int J Obes Relat Metab Disord 2000;24:1310–8.

122. Colditz G, Willett W, Stampfer M, London S, Segal M, Speizer F. Patterns of weight change and their relation to diet in a cohort of healthy women. Am J Clin Nutr 1990;51:1100–5.

123. Blackburn GL KB, Lavin PT, Keller SD, Whatley J. The effect of aspartame as part of a multidisciplinary weight-control program on short- and long-term control of body weight. Am J Clin Nutr 1997;65:409–18.

124. Raben A VT, Moller AC, Astrup A. Sucrose compared with artificial sweeteners: different effects on ad libitum food intake and body weight after 10 wk of supplementation in overweight subjects. Am J Clin Nutr. 2002;76:721–9.

125. Bolton-Smith C, Woodward M. Dietary composition and fat to sugar ratios in relation to obesity. Int J Obes Relat Metab Disord 1994(18):820–8.

126. Raben A, Macdonald I, Astrup A. Replacement of dietary fat by sucrose or starch: effects on 14 d ad libitum energy intake, energy expenditure and body weight in formerly obese and never-obese subjects. Int J Obes Relat Metab Disord 1997;21:846–59.

127. Tate D, Turner-McGrievy G, Lyons E, Stevens J, Erickson K, Polzien K, et al. Replacing caloric beverages with water or diet beverages for weight loss in adults: main results of the Choose

Healthy Options Consciously Everyday (CHOICE) randomized clinical trial. The American journal of clinical nutrition. 2012;95(3):555-63.

128. Peters J, Wyatt H, Foster G, Pan Z, Wojtanowski A, Vander Veur S, et al. The effects of water and non-nutritive sweetened beverages on weight loss during a 12-week weight loss treatment program. Obesity. 2014;22(6):1415-21.

129. French S, Lin B, Guthrie J. National trends in soft drink consumption among children and adolescents age 6 to 17 years: prevalence, amounts, and sources. J Am Diet Assoc 2003;103:1326–31.

130. Guthrie J, Morton J. Food sources of added sweeteners in the diets of Americans. J Am Diet Assoc. 2000;100:43–51.

131. Ebbeling C, Feldman H, Chomitz V, Gortmaker S, Osganian S, Ludwig D. A randomized trial of sugar-sweetened beverages and adolescent body weight. N Engl J Med 2012;367(15):1407-16.

132. De Ruyter J, Olthof M, Seidell J, Katan M. A trial of sugar-free or sugar-sweetened beverages and body weight in children. N Engl J Med. 2012;367(15):1397-406.

133. Gray D, Fujioka K. Use of relative weight and Body Mass Index for the determination of adiposity. J Clin Epidemiol. 1991;44(6):545–50.

134. National Institute of Health, National Heart, Lung, and Blood Institute. The Practical Guide: Identification, Evaluation, and Treatment of Overweight and Obesity in Adults. NIH Publication 2000:00-4084.

135. Douketis J, Maci eC, Thabane L, Williamson D. Systematic review of long-term weight loss studies in obese adults: clinical significance and applicability to clinical practice. Int J Obes 2005:29:1153.

136. Heymsfield S, Harp J, Reitman M. Why do obese patients not lose more weight when treated with low-calorie diets? A mechanistic perspective. Am J Clin Nutr 2007:85:346.

137. Mesejo A, Sanchez A, Arboleda Sanchez J. Guidelines for specialized nutritional and metabolic support in the critically-ill patient: update. Consensus SEMICYUC-SENPE: obese patient. Nutr Hosp. 2011;26(2):54-8.

138. Hall K, Sacks G, Chandramohan D. Quantification of the effect of energy imbalance on bodyweight. The Lancet 2011:378:826.

139. Freedman M, King J, Kennedy E. Popular diets: a scientific review Obes Res 2001;9(1):1S-40S.

140. Flechtner-Mors M, Ditschuneit H, Johnson T, Suchard M, Adler G. Metabolic and Weight Loss Effects of Long-Term Dietary Intervention in Obese Patients: Four-Year Results. Obesity Research. 2000;8(5):399-402.

141. Wing R, Hill J. Successful weight loss maintenance. Annual review of nutrition. 2001;21(1):323-41.

142. Brehm B, Seeley R, Daniels S, D'Alessio D. A randomized trial comparing a very low carbohydrate diet and a calorie-restricted low fat diet on body weight and cardiovascular risk factors in healthy women. The Journal of Clinical Endocrinology & Metabolism. 2003;88(4):1617-23.

143. Strychar I. Diet in the management of weight loss. CMAJ. 2006;174(1):56–63

144. Pereira M, Swain J, Goldfine A, Rifai N, Ludwig D. Effects of a low–glycemic load diet on resting energy expenditure and heart disease risk factors during weight loss. Jama. 2004;292(20):2482-90.

145. Catenacci V, Wyatt H. The role of physical activity in producing and maintaining weight loss. Nature Clinical Practice Endocrinology & Metabolism. 2007;3(7):518-29.

146. Villareal D, Chode S, Parimi N, Sinacore D, Hilton T, Armamento-Villareal R, et al. Weight loss, exercise, or both and physical function in obese older adults. New England Journal of Medicine. 2011;364(13):1218-29.

147. Jakicic J, Marcus B, Lang W, Janney C. Effect of exercise on 24-month weight loss maintenance in overweight women. Archives of internal medicine. 2008;168(14):1550-9.

148. Donnelly J, Blair S, Jakicic J, Manore M, Rankin J, Smith B. American College of Sports Medicine Position Stand. Appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults. Medicine and science in sports and exercise. 2009;41(2):459-71.

149. Turk M, Yang K, Hravnak M, Sereika S, Ewing L, Burke L. Randomized clinical trials of weight-loss maintenance: A review. The Journal of cardiovascular nursing. 2009;24(1):58.

150. Mekary R, Feskanich D, Hu F, Willett W, Field A. Physical activity in relation to Long-term weight maintenance after intentional weight loss in premenopausal women. Obesity. 2010;18(1):167-74.

151. Jakicic J, Marcus B, Gallagher K, Napolitano M, Lang W. Effect of exercise duration and intensity on weight loss in overweight, sedentary women: a randomized trial. Jama. 2003;290(10):1323-30.

152. World Health Organization WHO. Global report on diabetes. Geneva: 2016.

153. Harris M. Impaired glucose tolerance in the US population. Diabetes care. 1989;12(7):464-74.

154. Engelgau M, Geiss L, Saaddine J, Boyle J, Benjamin S, Gregg E, et al. The evolving diabetes burden in the United States. Annals of internal medicine. 2004;140(11):945-50.

155. Li C, Balluz L, Okoro C, Strine T, Lin J, Town M, et al. Surveillance of certain health behaviors and conditions among states and selected local areas: Behavioral Risk Factor Surveillance System, United States, 2009: US Department of Health and Human Services, Centers for Disease Control and Prevention; 2011.

156. Stumvoll M, Goldstein B, van Haeften T. Type 2 diabetes: principles of pathogenesis and therapy. The Lancet. 2005;365(9467):1333-46.

157. Mokdad A, Ford E, Bowman B. Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. JAMA. 2003;289(1):76-9.

158. Kan C, Silva N, Golden SH, Rajala U, Timonen M, Stahl D, et al. A systematic review and meta-analysis of the association between depression and insulin resistance. Diabetes care. 2013;36(2):480-9.

159. Tunceli K, Bradley C, Nerenz D, Williams L, Pladevall M, Elston Lafata J. The Impact of Diabetes on Employment and Work Productivity. Diabetes Care. 2005;28(11):2662-7.

160. Esteghamati A, Gouya M, Abbasi M, Delavari A, Alikhani S, Alaedini F, et al. Prevalence of Diabetes and Impaired Fasting Glucose in the Adult Population of Iran: National Survey of Risk Factors for Non-Communicable Diseases of Iran. Diabetes Care. 2008;31(1):96-8.

161. Haghdoost A, Rezazadeh Kermani M, Sadghirad B, Baradaran H. Prevalence of type 2 diabetes in the Islamic Republic of Iran: systematic review and meta-analysis. East Mediterr Health 2009.

162. Beck N, Henning G, Leif C. Metabolic and genetic characterization of prediabetic states. Sequence of events leading to non-insulin-dependent diabetes mellitus. Journal of Clinical Investigation. 1994;94(5):1714.

163. Kahn C. Ranting Lecture: Insulin action, dia-betogenes, and the cause of type II diabetes. 1994.

164. Robertson R, Bogardus C. Antagonist: diabetes and insulin resistance: philosophy, science, and the multiplier hypothesis. The Journal of laboratory and clinical medicine. 1995;125(5):560-5.

165. Li Y, Xu W, Liao Z, Yao B, Chen X, Huang Z, et al. Induction of long-term glycemic control in newly diagnosed type 2 diabetic patients is associated with improvement of β-cell function. Diabetes care. 2004;27(11):2597-602.

166. Fowler M. Diagnosis, classification, and lifestyle treatment of diabetes. Clinical diabetes. 2010;28(2):79-86.

167. DeFronzo R, Ferrannini E. Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes care. 1991;14(3):173-94.

168. Blunt B, Barrett-Connor E, Wingard D. Evaluation of fasting plasma glucose as screening test for NIDDM in older adults: Rancho Bernardo Study. Diabetes Care. 1991;14(11):989-93.

169. Kramer C, Araneta M, Barrett-Connor E. A1C and diabetes diagnosis: the Rancho Bernardo Study. Diabetes care. 2010;33(1):101-3.

170. Association. AD. Standards of medical care in diabetes—2010. Diabetes care. 2010;33(Supplement 1):S11-S61.

171. Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. The New England journal of medicine. 2002;346(6):393.

172. Zinman B, Ruderman N, Campaigne B, Devlin J, Schneider S. Physical activity/exercise and diabetes mellitus. Diabetes care. 2003;26:S73.

173. Klein S, Sheard NF, Pi-Sunyer X, Daly A, Wylie-Rosett J, Kulkarni K, et al. Weight Management Through Lifestyle Modification for the Prevention and Management of Type 2 Diabetes: Rationale and Strategies A statement of the American Diabetes Association, the North American Association for the Study of Obesity, and the American Society for Clinical Nutrition. Diabetes care. 2004;27(8):2067-73.

174. Sigal R, Kenny G, Wasserman D, Castaneda-Sceppa C, White R. Physical activity/exercise and Type 2 diabetes A consensus statement from the American Diabetes Association. Diabetes care. 2006;29(6):1433-8.

175. Boule N, Haddad E, Kenny G, Wells G, Sigal R. Effects of exercise on glycemic control and body mass in type 2 diabetes mellitus: a meta-analysis of controlled clinical trials. Jama. 2001;286(10):1218-27.

176. Franz M, Bantle J, Beebe C, Brunzell J, Chiasson J, Garg A, et al. Nutrition principles and recommendations in diabetes. Diabetes care. 2004;27:S36.

177. Bantle J, Swanson J, Thomas W, Laine D. Metabolic effects of dietary sucrose in type II diabetic subjects. Diabetes Care. 1993;16(9):1301-5.

178. Brand-Miller J, Petocz P, Colagiuri S. Meta-Analysis of Low–Glycemic Index Diets in the Management of Diabetes Response to Franz. Diabetes care. 2003;26(12):3363-4.

179. American Diabetes Association. Nutrition Recommendations and Interventions for Diabetes: A position statement of the American Diabetes Association. Diabetes Care. 2007;30(suppl 1):S48-S65.

180. Foster-Powell K, Holt S, Brand-Miller J. International table of glycemic index and glycemic load values: 2002. The American journal of clinical nutrition. 2002;76(1):5-56.

181. Fowler M. Diabetes treatment, part 1: diet and exercise. Clinical Diabetes. 2007;25(3):105-9.

182. Franz M, Bantle J, Beebe C, Brunzell J, Chiasson J, Garg A, et al. Evidence-based nutrition principles and recommendations for the treatment and prevention of diabetes and related complications. Diabetes care. 2002;25(1):148-98.

183. Payne M, Craig W, Williams A. Sorbitol is a possible risk factor for diarrhea in young children. Journal of the American Dietetic Association. 1997;97(5):532-4.

184. Mezitis N, Maggio C, Koch P, Quddoos A, Allison D, Pi-Sunyer F. Glycemic Effect of a Single High Oral Dose of the Novel Sweetener Sucralose in Patients With Diabetes. Diabetes Care. 1996;19(9):1004-5.

185. Gougeon R, Marliss E, Jones P, Pencharz P, Morais J. Effect of exogenous insulin on protein metabolism with differing nonprotein energy intakes in type 2 diabetes mellitus. International journal of obesity. 1998;22:250-61.

186. Gougeon R, Pencharz P, Sigal RJ. Effect of glycemic control on the kinetics of whole-body protein metabolism in obese subjects with non-insulin-dependent diabetes mellitus during iso- and hypoenergetic feeding. The American Journal of Clinical Nutrition. 1997;65(3):861-70.

187. Gannon M, Nuttall J, Damberg G, Gupta V, Nuttall F. Effect of Protein Ingestion on the Glucose Appearance Rate in People with Type 2 Diabetes The Journal of Clinical Endocrinology & Metabolism. 2001;86(3):1040-7.

188. Haffner S, Lehto S, Rönnemaa T, Pyörälä K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. New England journal of medicine. 1998;339(4):229-34.

189. Dennis EA, Flack KD, Davy BM. Beverage consumption and adult weight management: A review. Eating Behaviors. 2009;10(4):237-46.

190. Duffey KJ, Popkin BM. Shifts in Patterns and Consumption of Beverages Between 1965 and 2002. Obesity. 2007;15(11):2739-47.

191. Hu F. Resolved: there is sufficient scientific evidence that decreasing sugar-sweetened beverage consumption will reduce the prevalence of obesity and obesity-related diseases. obesity reviews. 2013;14(8):606-19.

192. Benton D. Can artificial sweeteners help control body weight and prevent obesity? Nutrition Research Reviews. 2005;18(01):63-76.

193. Williams CL, Strobino BA, Brotanek J. Weight control among obese adolescents: a pilot study. International journal of food sciences and nutrition. 2007;58(3):217-30.

194. Bellisle F, Altenburg de Assis M, Fieux B, Preziosi P, Galan P, Guy-Grand B, et al. Use of 'light'foods and drinks in French adults: biological, anthropometric and nutritional correlates. Journal of Human Nutrition and Dietetics. 2001;14(3):191-206.

195. Husoy T, Mangschou B, Fotland T, Kolset S, Nøtvik Jakobsen H, Tommerberg I, et al. Reducing added sugar intake in Norway by replacing sugar sweetened beverages with beverages containing intense sweeteners–a risk benefit assessment. Food and chemical toxicology. 2008;46(9):3099-105.

196. Guthrie JF, Morton JF. Food sources of added sweeteners in the diets of Americans. Journal of the American Dietetic Association. 2000;100(1):43-51.

197. Bellisle F, A D. Intense sweeteners, energy intake and the control of body weight. European Journal of Clinical Nutrition. 2007;61(6):691-700.

198. Mattes RD, Popkin BM. Nonnutritive sweetener consumption in humans: effects on appetite and food intake and their putative mechanisms. The American journal of clinical nutrition. 2009;89(1):1-14.

199. Morris D, Cuneo P, Stuart M, Mance M, Bell K, Puleo E, et al. High-intensity sweetener, energy and nutrient intakes of overweight women and men participating in a weight-loss program. Nutrition Research. 1993;13(2):123-32.

200. Fowler SP, Williams K, Resendez RG, Hunt KJ, Hazuda HP, Stern MP. Fueling the Obesity Epidemic? Artificially Sweetened Beverage Use and Long-term Weight Gain. Obesity. 2008;16(8):1894-900.

201. Popkin BM, Barclay DV, Nielsen SJ. Water and food consumption patterns of US adults from 1999 to 2001. Obesity research. 2005;13(12):2146-52.

202. Stookey J, Constant F, Popkin B, Gardner C. Drinking water is associated with weight loss in overweight dieting women independent of diet and activity. Obesity. 2008;16(11):2481-8.

203. Peters J, Beck J, Cardel M, Wyatt H, Foster G, Pan Z, et al. The effects of water and non-nutritive sweetened beverages on weight loss and weight maintenance: A randomized clinical trial. Obesity 2016;24(2):297-304.

204. Beck A, Beck R. Screening depressive patients in family practice. Postgrad Med. 1972;11:561-79.

205. Mason C, Katzmarzyk PT. Effect of the Site of Measurement of Waist Circumference on the Prevalence of the Metabolic Syndrome. The American journal of cardiology. 2009;103(12):1716-20.

206. American CoSM. ACSM's guidelines for exercise testing and prescription: Lippincott Williams & Wilkins; 2013.

207. American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 2014;37(Supplement 1):S81-S90.

208. Matthews DR HJ, Rudenski AS, Naylor BA, Treacher DF, Turner RC,. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28(7):412-19.

209. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma, Without Use of the Preparative Ultracentrifuge. Clinical Chemistry. 1972;18(6):499-502.

210. Bingham S. Limitations of the various methods for collecting dietary intake data. Annals of Nutrition and Metabolism. 1991;35(3):117-27.

211. Ireland J, van Erp-Baart A, Charrondiere U, Møller A, Smithers G, Trichopoulou A. Selection of a food classification system and a food composition database for future food consumption surveys. European Journal of Clinical Nutrition. 2002;56:S33-45.

212. Bingham SA. The dietary assessment of individuals; methods, accuracy, new techniques and recommendations1987.

213. Bingham S, Cassidy A, Cole T, Welch A, Runswick S, Black A, et al. Validation of weighed records and other methods of dietary assessment using the 24 h urine nitrogen technique and other biological markers. British Journal of Nutrition. 1995;73(04):531-50.

214. Maisey S, Loughridge J, Southon S, Fulcher R. Variation in food group and nutrient intake with day of the week in an elderly population. British Journal of Nutrition. 1995;73(03):359-73.

215. Jula A, Seppanen R, Alanen E. Influence of days of the week on reported food, macronutrient and alcohol intake among an adult population in south western Finland. European journal of clinical nutrition. 1999;53(10):808-12.

216. Wing R, Look AHEAD Research Group. Long term effects of a lifestyle intervention on weight and cardiovascular risk factors in individuals with type 2 diabetes: four year results of the Look AHEAD trial. Archives of internal medicine. 2010;170(17):1566.

217. Appel LJ, Champagne CM, Harsha DW, Cooper LS, E O. Die Auswirkungen von «Lifestyle»-Interventionen auf den Blutdruck. JAMA. 2003;289:2083-93.

218. Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. The New England journal of medicine. 2002;346(6):393.

219. Stroebele N, de Castro JM, Stuht J, Catenacci V, Wyatt HR, Hill JO. A small-changes approach reduces energy intake in free-living humans. Journal of the American College of Nutrition. 2009;28(1):63-8.

220. Madjd A, Taylor M, Delavari A, Malekzadeh R, Macdonald I, Farshchi H. Effects on weight loss in adults of replacing diet beverages with water during a hypoenergetic diet: a randomized, 24-wk clinical trial. The American Journal of Clinical Nutrition. 2015;102(6):1305-12.

221. Stellman S, Garfinkel L. Artificial sweetener use and one-year weight change among women. Prev Med. 1986;15(2):195-202.

222. Fowler S, Williams K, Hunt K, Resendez R, Hazuda H, Stern M, editors. Diet soft drink consumption is associated with increased

incidence of overweight and obesity in the San Antonio Heart Study. Diabetes; 2005: AMER DIABETES ASSOC 1701 N BEAUREGARD ST, ALEXANDRIA, VA 22311-1717 USA.

223. Ramage S, Farmer A, Apps Eccles K, McCargar L. Healthy strategies for successful weight loss and weight maintenance: a systematic review. Applied Physiology, Nutrition, and Metabolism. 2013;39(1):1-20.

224. Fowler SP, Williams K, Hazuda HP. Diet Soda Intake Is Associated with Long-Term Increases in Waist Circumference in a Biethnic Cohort of Older Adults: The San Antonio Longitudinal Study of Aging. Journal of the American Geriatrics Society. 2015;63(4):708-15.

225. Kraschnewski J, Boan J, Esposito J, Sherwood N, Lehman E, Kephart D, et al. Long-term weight loss maintenance in the United States. International journal of obesity 2010;34(11):1644-54.

226. Klein S, Sheard NF, Pi-Sunyer X, Daly A, Wylie-Rosett J, Kulkarni K, et al. Weight Management Through Lifestyle Modification for the Prevention and Management of Type 2 Diabetes: Rationale and Strategies: A statement of the American Diabetes Association, the North American Association for the Study of Obesity, and the American Society for Clinical Nutrition. Diabetes Care. 2004;27(8):2067-73.

227. Nettleton J, Lutsey P, Wang Y, Lima J, Michos E, Jacobs D. Diet soda intake and risk of incident metabolic syndrome and type 2 diabetes in the Multi-Ethnic Study of Atherosclerosis (MESA). Diabetes Care. 2009;32(4):688-94.

228. Mackenzie T, Brooks B, O'Connor G. Beverage Intake, Diabetes, and Glucose Control of Adults in America. Annals of Epidemiology. 2006;16(9):688-91.

229. James PT. Obesity: the worldwide epidemic. Clinics in dermatology. 2004;22(4):276-80.

230. Millen B, Lichtenstein A, Abrams S. Scientific Report of the 2015 Dietary Guidelines Advisory Committee. Washington (DC): Department of Health and Human Services. 2015.

231. Heitmann B, Lissner L, Osler M. Do we eat less fat, or just report so? International journal of obesity. 2000;24(4):435-42.

232. Lissner L, Heitmann BL, Lindroos AK. Measuring intake in free-living human subjects: a question of bias. Proceedings of the Nutrition Society. 1998;57(02):333-9.

234. McCrickerd K, Chambers L, Yeomans MR. Fluid or Fuel? The Context of Consuming a Beverage Is Important for Satiety. PLoS ONE. 2014;9(6):e100406.

235. Suez J, Korem T, Zeevi D, Zilberman-Schapira G, Thaiss CA, Maza O, et al. Artificial sweeteners induce glucose intolerance by altering the gut microbiota. Nature. 2014;514(7521):181-6.

236. Malo M, Moaven O,Muhammad N,Biswas B. Intestinal alkaline phosphatase promotes gut bacterial growth by reducing the concentration of luminal nucleotide triphosphates. American Journal of Physiology-Gastrointestinal and Liver Physiology 2014; DOI: 10.1152/ajpgi.00357.2013

Appendices

Appendix-1

MEDICAL HISTORY AND SCREENING FORM

General Information

First name: Surname:

Date of Birth:

Sex:

☐ Male ☐ Female

Marital Status:

☐ Single ☐ Married

Education:

☐ Grade School ☐ High School

☐ Graduate School ☐ Degree ________________

Occupation:

Contact Information

Tel. No: Mobile No:

Fax. No:

Email:

Address:

Chief Complaint (the major health problem or concern):

Its time course:

More details:

Anthropometric measurements and Blood pressure

Weight: ….kg Height: ……m

Waist Circumference: ….Cm BP:……mmHg

Family medical history

Obesity Diabetes Hypercholesterolemia Hypertriglyceridemia

Cardiovascular Diseases Fatty Liver

Cancer

Past Medical History

Check those questions to which your answer is yes (leave others blank).

Diabetes or abnormal blood-sugar tests

Hypercholesterolemia

Hypertriglyceridemia

Fatty Liver

Hepatitis

Gallbladder stones

Heart attack if so, how many years ago? _________

Rheumatic Fever

Diseases of the arteries

Varicose veins

Arthritis of legs or arms

Phlebitis (inflammation of a vein)

Dizziness or fainting spells

Epilepsy or seizures

Stroke

Nervous or emotional problems

Anemia

Hypothyroidism

Hyperthyroidism

Renal Failure

Renal stones

Pneumonia

Bronchitis

Asthma

Abnormal chest X-ray

Other lung disease

Injuries to back, arms, legs or joint

Broken bones

Limitation in physical activity

Other diseases:

☐ Food intolerance? If yes which foods?

☐ Food allergy? If yes which foods?

☐ Taking any medication? If yes what?

List any prescription medications you are now taking:

……………………………………………………………………………

List any self-prescribed medications, dietary supplements, or vitamins you are now taking:

…………………………………………………………………………….

☐ Having any medical condition?

Comments:

List any other medical or diagnostic test you have had in the past two years:

List hospitalizations, including dates of and reasons for hospitalization:

List any drug allergies:

For Women only.

Do you have:

☐ Menstrual period problems?

○ Irregular menstruation

○ Lack of menstruation

☐ Android Hair Loss

Abnormal Hair growth (hirsutism)

Pregnancy History

☐ Children? If yes, how many?

☐ Abortion:

☐ Significant childbirth

☐ Urine loss when you cough, sneeze or laugh?

☐ Are you on any type of hormone replacement therapy?

☐ Are pregnant?

☐ Are you breastfeeding?

Personal Habits

Have you ever smoked cigarettes, or a pipe?

☐Yes ☐No

If you did or now smoke cigarettes, how many per day?

____

____

If you did or now smoke a pipe, how many pipefuls a day?

____

If you have stopped smoking, when was it? __________________

If you now smoke, how long ago did you start? ________________

Do you ever drink alcoholic beverages? ☐ Yes☐No

If yes, what is your approximate intake of these beverages?

Exercise and Physical activity

-Do you exercise regularly?

☐ Yes ☐ No

-If yes what type of exercise? Duration of each session? How many times/week?

-Are you walking regularly?

☐ Yes ☐ No

If yes how often?

Did you had Professional sports in the past?

Diet History

Have you ever had diet before?

☐ Yes ☐ No

If yes, when was the last time?

The maximum weight loss with diet?

Surgical intervention for weight loss

Did you ever take any medication for weight loss? If yes, what?

Did you ever had Weight loss surgery?

If yes which type surgery and when you did?

How much weight loss you had with this intervention?

History of Physical and diagnostic examination

Date of last complete physical examination: ______________________

☐ Normal ☐ Abnormal ☐ Never ☐ Can’t remember

Date of last chest X-ray: ____

☐ Normal ☐ Abnormal ☐ Never ☐ Can’t remember

Date of last electrocardiogram (ECG): ________________

☐ Normal ☐ Abnormal ☐ Never ☐ Can’t remember

Date of last dental checkup: ________________________________

☐ Normal ☐ Abnormal ☐ Never ☐ Can’t remember

Date of last laboratory checkup: ______________________________

☐ Normal ☐ Abnormal ☐ Never ☐ Can’t remember

Appendix-2

Four-Day Food Diary Record

Name: ____

Dates of recorded intake: ________________________________

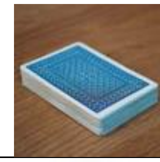
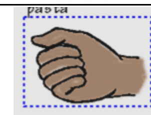
Instructions for Keeping Your Four-Day Food Record

- Please keep your Four-day food record for four days.
- The days should include three weekdays and one weekend day.
- Select days that closely resemble your usual eating habits.
- Each time you eat or drink anything (meals, snacks, etc.) during the four days, write down what and how much was served and what and how much was eaten.
- To measure how much was eaten, use a set of **measuring cups and spoons** to help estimate amounts. Also see the examples below to estimate portion sizes.
- Note if food choices are homemade or purchased. Please include brand names whenever possible.

Amounts and Conversions

1/4 cup = 50 ml or 4 Tablespoons
1/3 cup = 75 ml or 5 1/2 Tablespoons
1/2 cup = 125 ml or 8 Tablespoons
2/3 cup = 150 ml or 10 1/2 Tablespoons
3/4 cup = 175 ml or 12 Tablespoons
1 cup = 250 ml or 16 Tablespoons
1 oz = 1 slice of processed cheese or lunchmeat

How to Estimate Your Portion Size

Meat Three 85 gram of meat are about the size and thickness of a deck of playing cards or an audiotape cassette.	
Fruit A medium apple or peach is about the size of a tennis ball.	
Grains One cup of rice or pasta is about the size of your fist.	
Cheese One ounce of cheese is about the size of four dice.	

Three-Day Food Record Checklist

Beverages	What kind of milk? Homo, 2%, 1%, skim, other. Was it fruit juice or fruit beverage or drink?
Breads	Did you spread on butter or margarine?
Cereal	Did you add milk? Did you add sugar or fruit?
Dairy	What brand or kind of yogurt? What brand or kind of cheese?
Vegetables	Was it raw or cooked? Was it fresh, frozen or canned? Did you add any butter, margarine or sauce?
Fruit	Was it a small, medium or large fruit? Was it fresh, frozen or canned?
Grains	Did you add any butter, margarine, peanut butter, jam or honey? Was it a half or whole sandwich? Was it a small or large muffin or bagel?
Fish	Was your canned fish packed in water or oil How did you cook your fish?
Meats	How did you cook your meat? What kind of cut was it e.g. chicken leg or chicken breast?
Soups	Was your soup prepared with milk, water or cream?
Restaurants	What restaurant was it?
Packaged food	What brand was it?

Sample Menu

Day 1: Tuesday, May 12, 2013				
Time of Meal or Snack	**Type of Food or Beverage Offered**	**Amount Eaten**	**Method of Preparation or Brand**	**Comments** (e.g. amount of food served, too tired to eat)
Breakfast	Cereal	½ cup	Honey Nut Cheerios	
	Milk 2%	½ cup	Damdaran	On cereal
	Banana	½ med		
AM Snack	Animal Crackers	10	Christie	
	Apple juice	113gram	apple juice	
Lunch	Grilled cheese sandwich			
	Whole wheat bread	1 slice	Senan	No crusts
	Cheese slice	1 slice	kibi slices	
	Butter on bread	1 Tbsp		
	Yogurt – strawberry	75 ml		
	Milk	½ cup	2%	
PM Snack	Granola bar	1 bar – 35 g		Ate half of it
Dinner	Chicken fingers	1 ½		
	French fries	10	Chitoos	
	Honey	2 Tbsp		For dipping
	Ketchup	2 Tbsp	Heinz	
	Carrots	½ medium	Raw, cut in sticks	
	Milk	½ cup	2%	
Evening Snack	Ice cream	1 cup	Chocolate Mihan	

Was this day's intake considered: [ ] Poor [X] Average [ ] Very Good

Day 1	**Date:**			
Time of Meal or Snack	**Type of Food or Beverage Offered**	**Amount Eaten**	**Method of Preparation or Brand**	**Comments** (e.g. amount of food served, too tired to eat)
Breakfast				
AM Snack				
Lunch				
PM Snack				
Dinner				
Evening Snack				

Was this day’s intake considered: [ ] Poor [ ] Average [ ] Very Good

Day 2	Date:			
Time of Meal or Snack	**Type of Food or Beverage Offered**	**Amount Eaten**	**Method of Preparation or Brand**	**Comments** (e.g. amount of food served, too tired to eat)
Breakfast				
AM Snack				
Lunch				
PM Snack				
Dinner				
Evening Snack				

Was this day’s intake considered: [ ] Poor [ ] Average [ ] Very Good

Day 3	**Date:**			
Time of Meal or Snack	**Type of Food or Beverage Offered**	**Amount Eaten**	**Method of Preparation or Brand**	**Comments** (e.g. amount of food served, too tired to eat)
Breakfast				
AM Snack				
Lunch				
PM Snack				
Dinner				
Evening Snack				

Was this day's intake considered: [ ] Poor [ ] Average [ ] Very Good

Day 4	Date:			
Time of Meal or Snack	**Type of Food or Beverage Offered**	**Amount Eaten**	**Method of Preparation or Brand**	**Comments** (e.g. amount of food served, too tired to eat)
Breakfast				
AM Snack				
Lunch				
PM Snack				
Dinner				
Evening Snack				

Was this day’s intake considered: [ ] Poor [ ] Average [ ] Very Good

Appendix-3

Lifestyle and Eating Habits Questionnaire

Name:

File No:

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1. What time you do usually wake up weekdays?
2. What time you do usually wake up weekend?
3. What time do you usually sleep at night weekdays?
4. What time do you usually sleep at night weekends?
5. How many hours do you usually sleep per day?
6. How many hours do you usually watching television per day?
7. How many hours do you usually using computers per day?
8. How much time do you usually spend using mobile apps per day?
9. Do you usually eat fast? Yes No
10. How many minutes usually each meal take to eat?
11. How often do you eat Fast food? What kind of fast food do you eat usually?
12. How often do you eat the traditional restaurant foods (such as kebab, chicken kebab, etc.)? What do you usually eat at restaurants?
13. How often do you eat ready-meals per week?
14. How often do you eat rice? How many spoon of rice do you eat per each serving?
15. Is there any food that you dislike? If yes what?
16. Do you have food allergy? If yes to which foods do you have

allergy?

17. Which food do you like more?

18. Do you use dairy products? If yes, how many times a day? (Low-fat or high-fat)

Appendix-4

The Physical Activity Readiness Questionnaire (PAR-Q)

Being physically active is very safe for most people. Some people, however, should check with their doctors before they increase their current level of activity. The PAR-Q has been designed to identify the small number of adults for whom physical activity may be inappropriate or those who should have medical advice concerning the type of activity most suitable for them.

Answer yes or no to the following questions:

1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?
2. Do you feel pain in your chest when you do physical activity?
3. In the past month, have you had chest pain when you were not doing physical activity?

1. Do you lose your balance because of dizziness or do you ever lose consciousness?
2. Do you have a bone or joint problem that could be made worse by a change in your physical activity?
3. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?
4. Do you know of any other reason why you should not do physical activity?

Appendix-5

Beck's Depression Inventory Questionnaire

Please place a (√) in front of which applies best to each of the numbered statements. All of the results will be strictly confidential. Most of the questions directly related to your health history situations. Please answer each question carefully, thank you.

1. (Sadness)

0 I do not feel sad.

1 I feel sad.

2a I am sad all the time and I can't snap out of it.

2b I am so sad and unhappy that it quite painful.

3 I am so sad and unhappy that I can't stand it.

2. (Pessimism)

0 I am not particularly discouraged about the future.

1 I feel discouraged about the future.

2a I feel I have nothing to look forward to.

2b I feel I won’t ever get over my troubles.

3 I feel the future is hopeless and that things cannot improve.

3. (Sense of failure)

0 I do not feel like a failure.

1 I feel I have failed more than the average person.

2a I feel I have accomplished very little that is worthwhile or

That means anything

2b as I look back on my life, all I can see is a lot of failures.

3 I feel I am a complete failure as a person.

4. (Dissatisfaction)

0 I am not particularly dissatisfied.

1a I feel bored most of the time.

1b I don't enjoy things the way I used to.

2 I don't get real satisfaction out of anything anymore.

3 I am dissatisfied or bored with everything.

5. (Guilt)

0 I don't feel particularly guilty.

1 I feel bad or unworthy a good part of the time.

2a I feel quite guilty.

2b I feel bad or unworthy particularly all the time now.

3 I feel guilty all of the time.

6. (Expectation of punishment)

0 I don't feel I am being punished.

1 I have a feeling that something bad may happen to me.

2 I feel I am being punished or will be punished.

3a I feel I deserve to be punished.

3b I want to be punished

7. (Self-dislike)

0 I don't feel disappointed in myself.

1a I am disappointed in myself.

1b I don’t like myself.

2 I am disgusted with myself.

3 I hate myself.

8. (Self-accusation)

0 I don't feel I am any worse than anybody else.

1 I am critical of myself for my weaknesses or mistakes.

2 I blame myself all the time for my faults.

3 I blame myself for everything bad that happens.

9. (Suicidal ideas)

0 I don't have any thoughts of harming myself.

1 I have thoughts of harming myself, but I would not carry them out.

2a I feel I would be better off dead.

2b I feel my family would be better off if I were dead.

3a I have definite plans about committing suicide.

3b I would kill myself if I could.

10. (Crying)

0 I don't cry any more than usual.

1 I cry more now than I used to.

2 I cry all the time now.

3 I used to be able to cry, but now I can't cry even though I want to.

11. (Irritability)

0 I am no more irritated by things than I ever was.

1 I get annoyed or irritated more easily than usual.

2 I feel irritated all the time.

3 I don’t get irritated at all at the things that used to irritate me.

12. (Social withdrawal)

0 I have not lost interest in other people.

1 I am less interested in other people than I used to be.

2 I have lost most of my interest in other people and have little feeling for them.

4 I have lost all of my interest in other people and don’t care about Them.

13. (Indecisiveness)

0 I make decisions about as well as I ever could.

1 I try put off making decisions.

2 I have greater difficulty in making decisions more than I used to.

3 I can't make decisions at all anymore.

14. (Body image change)

0 I don't feel that I look any worse than I used to.

1 I am worried that I am looking old or unattractive.

2 I feel there are permanent changes in my appearance that make me look unattractive

3 I believe that I am ugly or repulsive looking.

15. (Work retardation)

0 I can work about as well as before.

1a It takes an extra effort to get started at doing something.

1b I don’t work as well as I used to.

2 I have to push myself very hard to do anything.

3 I can't do any work at all.

16. (Insomnia)

0 I can sleep as well as usual.

1 I wake up more tried in the morning than used to.

2 I wake up 1-2 hours earlier than usual and find it hard to get back to sleep.

3 I wake up early every day and can’t get more than 5 hours sleep.

17. (Fatigability)

0 I don't get any more tired than usual.

1 I get tired more easily than I used to.

2 I get tired from doing almost anything.

3 I am too tired to do anything.

18. (Anorexia)

0 My appetite is no worse than usual.

1 My appetite is not as good as it used to be.

2 My appetite is much worse now.

3 I have no appetite at all anymore.

19. (Weight loss)

0 I haven't lost much weight, if any, lately.

1 I have lost more than five pounds.

2 I have lost more than ten pounds.

3 I have lost more than fifteen pounds.

20. (Somatic preoccupation)

0 I am not more concerned about my health than usual.

1 I am concerned about aches, pains, upset stomach, or constipation.

2 I am so concerned with how I feel or what I feel that its

hard to think of much else.

3 I am completely absorbed in what I feel.

21. (Loss of libido)

0 I have not noticed any recent change in my interest in sex.

1 I am less interested in sex than I used to be.

2 I have almost no interest in sex.

3 I have lost interest in sex completely.

Appendix-6

Beverage intake questionnaire

Beverage Questionnaire

Instructions:
In the past month, please indicate your response for each beverage type by marking an "X" in the bubble for "how often" and "how much each time"
1) Indicate how often you drank the following beverages, for example, you drank 5 glasses of water per week, therefore mark 4-6 times per week
2) Indicate the approximate amount of beverage you drank each time, for example, you drank 1 cup of water 2 times per day, therefore mark 1 cup under "how much each time"

Subject ID ____________________

Date ____________________

	HOW OFTEN (MARK ONE)							HOW MUCH EACH TIME (MARK ONE)				
Type of Beverage	**Never or less than 1 time per week (go to next beverage)**	**1 time per week**	**2-3 times per week**	**4-6 times per week**	**1 time per day**	**2+ times per day**	**3+ times per day**	**Less than 6 fl oz (3/4 cup)**	**8 fl oz (1 cup)**	**12 fl oz (1 1/2 cups)**	**16 fl oz (2 cups)**	**More than 20 fl oz (2 1/2 cups)**
Water	O	O	O	O	O	O	O	O	O	O	O	O
100% Fruit Juice	O	O	O	O	O	O	O	O	O	O	O	O
Sweetened Juice Beverage/Drink (fruit ades, lemonade, punch, Sunny Delight)	O	O	O	O	O	O	O	O	O	O	O	O
100% Vegetable Juice (V8, etc.)	O	O	O	O	O	O	O	O	O	O	O	O
Whole Milk	O	O	O	O	O	O	O	O	O	O	O	O
Reduced Fat Milk (2%)	O	O	O	O	O	O	O	O	O	O	O	O
Low Fat/Fat Free Milk (Skim, 1%, Buttermilk, Soymilk)	O	O	O	O	O	O	O	O	O	O	O	O
Soft Drinks, Regular	O	O	O	O	O	O	O	O	O	O	O	O
Diet Soft Drinks/Artificially Sweetened Drinks (Crystal Light)	O	O	O	O	O	O	O	O	O	O	O	O
Sweetened Tea	O	O	O	O	O	O	O	O	O	O	O	O
Coffee, with cream and/or sugar (includes non-dairy creamer)	O	O	O	O	O	O	O	O	O	O	O	O
Tea or Coffee, black, with/without artificial sweetener (no cream or sugar)	O	O	O	O	O	O	O	O	O	O	O	O
Non-alcoholic or Light Beer	O	O	O	O	O	O	O	O	O	O	O	O
Beer, Ales, Wine Coolers	O	O	O	O	O	O	O	O	O	O	O	O
Hard Liquor (shots, rum, tequila, etc.)	O	O	O	O	O	O	O	O	O	O	O	O
Mixed Alcoholic Drinks (daiquiris, margaritas, etc.)	O	O	O	O	O	O	O	O	O	O	O	O
Wine (red or white)	O	O	O	O	O	O	O	O	O	O	O	O
Meal Replacement Shakes/Protein Drinks (Slimfast, shakes, etc.)	O	O	O	O	O	O	O	O	O	O	O	O
Energy Drinks (Red Bull, Rockstar, Full Throttle, etc.)	O	O	O	O	O	O	O	O	O	O	O	O
Other (list):	O	O	O	O	O	O	O	O	O	O	O	O
Other (list):	O	O	O	O	O	O	O	O	O	O	O	O

Virginia Polytechnic Institute and State University, 2008

Appendix-7

Report at the end of two weeks (diet adherence questionnaire):

Name:

File No:

1. The list of foods that you desire to have in your next diet:

2. The list of fruits that you desire to have in your next diet:

3. The List of non-compliance (regarding eating time and physical activity):

4. The list of disobedience (regarding recommended diet)?

5. What is bothering you in the diet?

Comments: