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Moderate sugar-sweetened beverage consumption impairs low density lipoprotein metabolism in healthy young men: A randomized, controlled, double-blinded study

MASTERARBEIT

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vorgelegt von
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Contents

1. Abstract

Background: Sugar-sweetened beverage consumption in high quantities impairs lipid metabolism and therefore causes metabolic disorders in obese subjects. However, the effect of sugar sweetened beverages in lower doses on normal weight healthy subjects remains less clear.

Objective: The aim of the study was to investigate the impact of sugar-sweetened beverage consumption over 7 weeks on low density lipoprotein particle size and distribution.

Design: 96 normal weight, healthy young male subjects were included in a randomized, double blind, monocentric, controlled nutritional trial and were randomly assigned to 4 intervention groups. Over a period of 7 weeks, subjects were instructed to consume on a daily basis 600ml of sugar-sweetened beverages containing either 80g fructose, 80g glucose or 80g sucrose, whereas a control group was advised to abstain completely from sugar-sweetened beverages.

Results: Small low density lipoprotein particles (22-25.6nm diameter) increased and large low density lipoprotein particles (25.6-28.5nm diameter) decreased after sugar-sweetened beverage consumption compared with baseline ($p = 0.04$). The sucrose containing intervention decreased the largest low density lipoprotein particles (27.2-28.5nm diameter) significantly ($p = 0.039$).

Conclusion: This study shows that even moderate consumption of sugar-sweetened beverages leads to adverse effects on low density lipoprotein metabolism in normal weight, healthy young men.

2. Abbreviations

Abbreviations	***Full String***
Acyl-CoA	Acyl coenzyme A
ASAT	Aspartate aminotransferase
ALAT	Alanine transaminase
BMI	Body mass index
CK	Creatine kinase
CPT I	Carnitine palmitoyl transferase I
EDTA	Ethylene diamine tetraacetic acid
LDL	Low density lipoprotein
Malonyl-CoA	Malonyl coenzyme A
PPARα	Peroxisome proliferator activated receptor α
SD	Standard deviation
sdLDL	Small dense low density lipoprotein
SSB	Sugar-sweetened beverage
VLDL	Very low density lipoprotein

3. Introduction

3.1. Background

Strong epidemiological evidence suggests a causal relation between fructose consumption and metabolic disorders (1, 2). Over the past decades, sugar-sweetened beverages (SSBs) turned into the leading source of added sugars in the United States (3). After the introduction of high-fructose corn syrup as a beverage sweetener, there was simultaneously an increase in levels of overweight and obesity in all population groups observed (4). Additionally, caloric sweetener consumption has been associated with dyslipidaemia, known to increase the risk of cardiovascular diseases (5, 6). In fact, regular consumption of SSBs is associated with an increased risk of coronary heart disease (7, 8). SSB consumption was also found to be associated with an elevated risk of developing type 2 diabetes (9).

Moreover, animal studies showed, that fructose, in contrast to glucose, induces dyslipidaemia and insulin resistance (10, 11, 12, 13, 14). A more recent study in overweight humans showed fructose-sweetened, but not glucose-sweetened, beverages to increase visceral adiposity and lipids and also to decrease insulin sensitivity (15). In a cross-sectional study in schoolchildren an association between high fructose intake with decreased low density lipoprotein (LDL) particle size was found (2). Secondary, there is an association between smaller LDL particle size and the metabolic syndrome and may be an early marker for atherosclerosis and type 2 diabetes (16, 17, 18).

There are several important differences between the metabolism of fructose and glucose (4, 19). First of all, fructose as a highly lipogenic substrate increases lipid synthesis in various ways (15). After consumption of fructose, it gets rapidly cleared from the blood plasma and metabolized by the enzyme fructokinase in the liver (20, 21). Furthermore, the rate of fructose being absorbed from the portal blood circulation by the liver is higher than the rate of glucose being absorbed (19). While the enzymes phosphorylating glucose (phosphofructokinase and glucokinase) are regulated by the energy status of the cell and insulin, fructokinase is not and bypasses major regulatory steps of glycolysis (20, 21). Consequently, it leads to an increased production of triose phosphates, which then leads to increased de novo synthesis of Acyl coencyme A (acyl-CoA), triglycerides and therefore resulting in higher levels of very low density lipoprotein (VLDL) synthesis and secretion from the liver to the blood stream (20, 21). Alternatively, triose phosphates generated from fructose can be oxidized by the hepatocyte, converted to lactate or enter the gluconeogenesis (13).

In addition, high levels of VLDL result in higher concentrations of atherogenic small dense low density lipoprotein (sdLDL) due to more extensive lipid remodelling (11, 15, 22). On the other hand, fructose decreases lipid degradation via suppression of beta-oxidation (23). After high

fructose diets, peroxisome proliferator activated receptor α (PPARα) dependent lipid oxidation genes were downregulated and PPARα were reduced in their activity (23). These changes were not observed after high glucose consumption (24). Additionally, hepatic de novo lipogenesis slows down beta-oxidation by producing malonyl coencyme A (malonyl-CoA), which inhibits carnitine palmitoyl transferase I (CPT I) and therefore reduces the entry of fatty acids into the mitochondria (22). In short, fructose induces lipid synthesis and therefore causes not only higher levels of VLDL synthesis and secretion, but also enhances conversion from VLDL to atherogenic sdLDL (11, 15, 20, 21, 22).

3.2. Rationale for current study

Previous studies already investigated effects of fructose on lipid metabolism, but mostly in very high doses of pure fructose have been utilized, providing up to 25 - 60% of total daily energy, which does not necessarily reflect the amount of fructose currently encountered in the human diet (22). There have also been several studies on obese subjects, but the effect of fructose on normal weight, healthy subjects remains less clear (15, 25). Besides, it is important to note, that fructose is mostly coingested with glucose via sucrose or industrial blends in form of corn syrup (22). Therefore, it is necessary not only to examine the effects of fructose on lipid metabolism, but also to separate them from the effects of glucose. The consequences of SSB consumption in different doses of fructose, glucose or sucrose on lipid metabolism of healthy young male subjects was already examined by Aeberli et al. (26), showing the negative impact of SSBs in healthy young men on lipid metabolism. This study had two major limitations: Firstly, the interventions with SSBs only lasted 3 weeks and this may not have been long enough to detect significant effects and secondly, there was the possibility of carryover effects between the different interventions having a washout period of only ≥4 weeks (26).

The aim of the current study was to investigate the relations between dietary intake of fructose, glucose or sucrose (in amounts comparable to the ones in commercially sweetened beverages) and LDL size alternations. The major limitations of the Aeberli et al. study (26) (as just described) were corrected in the present study by extending the period of intervention to 7 weeks and by appointing to each intervention a different subject in order to avoid a carryover effect. Based on literature, the main hypotheses were:

- Consumption of SSBs causes a reduction in LDL particle size;
- Fructose-sweetened beverages reduce LDL particle size more than glucose-sweetened beverages.

In conclusion, the objective of the current study was to investigate the effect of moderate SSB consumption on LDL particle size alternations in healthy young men.

4. Material and Methods

4.1. Study design

In this randomized, double blind (relating to SSB intervention), monocentric, controlled nutritional study 96 subjects were included. The investigation of LDL particle size was a secondary outcome of a larger project investigating changes in fatty acid synthesis due to SSB consumption as primary outcome. The subjects were equally divided into 4 groups of 24 subjects each. Each of the 4 groups obtained in parallel a different intervention after a baseline examination. Therefore a randomization procedure was used to avoid a bias. Randomization lists were prepared by the hospital pharmacy (Kantonsapotheke Zürich). In general, the different interventions lasted 7 weeks and subjects were examined in visits at the University Hospital Zurich after week 5, week 6 and week 7. All subjects were examined in a time interval of April 2013 to December 2015.

During 3 out of 4 interventions, the subjects were supplied with SSBs containing either 80g fructose per day, 80g glucose per day or 80 g sucrose per day. Subjects were instructed to drink 3 times a bottle per day, each contained 200ml of water mixed with 26.6g of fructose, glucose or sucrose. All bottles were produced by Molkerei Biedermann AG in Bischofszell/TG, Switzerland. Subjects of the 4th intervention, serving as control group, abstained completely from SSBs. The different interventions were randomly assigned to the subjects. Both the study team and subjects were blinded concerning the intervention with SSBs.

4.2. Subjects

96 healthy, non-smoking male volunteers (age between 18 and 30 years, body mass index (BMI) between 19 and 24 kg/m^2) of European ancestry were included in the study. The subjects were recruited in student facilities using posters and newspaper advertisements approved by the local ethics committee. Only male members were included, since there is evidence of a different metabolic effect of fructose on male and female subjects (27). The following criteria led to an exclusion of the subjects:

- Acute or chronic infections, malignant disease, renal, hepatic (more than two-fold increased transaminases), pulmonary, neurological (epilepsy) or psychiatric diseases, manifested atherosclerosis, or any other disease precluding participation in the study.
- Diabetes
- Known alcohol, substance or drug abuse, concomitant medication

- More than three hours of physical exercise per week
- Consumption of more than 1 times 3 dl SSB daily
- Subjects likely to fail to comply with the study protocol
- Subjects who participate to another clinical study or have participated to a clinical study in the past 6 months
- Subjects who have donated blood or blood components in the past 6 months
- Subjects who do not give informed consent

4.3. Study visits

The study consisted of a screening visit followed by 4 examination visits. In the screening visit, all subjects were thoroughly informed about the study and received a proband's information sheet. After obtaining the written informed consent, anamnestic data about the medical history, medication and systemic diseases were collected. Then, morphometric measurements (body weight, height, BMI) were done and blood samples were taken in order to measure haemoglobin, haematocrit, fasting glucose, plasma lipids liver and renal function (aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), creatine kinase (CK), creatinine) and to screen for HIV antibodies. All subjects meeting the inclusion criteria without violating the exclusion criteria were included in the study. In general, subjects were informed not to drink SSBs 4 weeks before the baseline visit. They were instructed to record each time all food and drinks consumed 3 days prior to a next visit. In addition, they were told not to eat anything for at least 12 hours, not to drink alcohol or coffein containing drinks, not to smoke and not to do excessive sports during 24 hours prior to the next visit. The evening before a visit, they should abstain from fatty meals.

The examination consisted in total of 4 visits (a baseline visit and a visit after 5, 6 and 7 weeks of intervention) at the University Hospital Zurich. In every visit, subjects were provided with SSBs and were instructed to drink 3 times a day at every meal one SSB containing 200ml each until the next visit (except for the last visit after 7 weeks). Furthermore, the overnight fasting blood samples for LDL analysis were drawn in a sitting position at 7^{00} to 8^{00} am in all visits. In addition to blood samples, other parameters not relevant to the study described here were measured. Subjects were questioned about their sense of wellbeing and about adverse events. Also they had to submit their record of eating habits, their empty bottles of SSBs and not consumed bottles of SSBs (except the baseline visit). For the calculation of LDL particle size and fractions after the intervention, mean values of the 3 visits after 5, 6 and 7 weeks of intervention were generated in order to obtain robust results.

4.4. Laboratory analysis

Laboratory analysis was done in the endocrinology laboratory at the University Hospital Zurich. Ethylene diamine tetraacetic acid (EDTA) tubes were used for the overnight fasting blood samples and were centrifuged within 30 min. Plasma for LDL size analysis was frozen and stored at -80°C. Nondenaturating polyacrylamide gradient gel electrophoresis was performed in 2-16% polyacralamide gradient gels at 10-14°C. As described in (28, 29, 30), gel electrophoresis was performed for 24h at 125 V in tris borate buffer (pH 8.3). In a solution of red O in 60% ethanol at 55°C gels were fixed and stained for lipids. Gels were photographed with a Canon G3 digital camera. The pictures were analysed using the LDL particle analyser program (University Hospital Zurich) as follows: For each absorbance peak, migration distance was determined and the corresponding molecular diameter calculated. The calibration curve was generated from the migration distance of standardised substances of known diameter, namely apoferritin and thyroglobulin (HMWStd, Pharmacia, Piscataway, NJ) and carboxylated latex beads (Duke scientific, Palo Alto, CA) with a molecular diameter of 122 Å, 170 Å and 380 Å. There were also lipoprotein calibrator of previously determined particle size used. As previously described in (29), LDL subclass distribution in relation to total LDL was calculated.

4.5. Statistical analysis

Data are described as mean ± standard deviation (SD). Changes of parameter within groups were analysed with the Wilcoxon test. Changes between groups were analysed with the Mann-Whitney or Kruskal-Wallis test. In addition to particle analysis of subgroups, there was an analysis of the interventions as a whole. P-values < 0.05 were considered being significant.

4.6. Ethics

The study protocol was approved by the ethics committee of the Kanton Zurich (Kantonale Ethikkomission Zürich, KEK-ZH Nr. 2012-0160). All subjects were thoroughly informed about the study and received a proband's information sheet. Subjects signed an informed consent before the enrolment.

5. Results

5.1. Baseline characteristics

Out of 96 subjects initial assigned, a total of 94 subjects completed the study. 2 Subjects did not finish the study. Important characteristics of the subjects are summarized in **Table 1**. There were significant differences among the intervention groups in age (p = 0.012), height (p = 0.039) and BMI (p = 0.006) despite randomisation.

	Intervention				**p**
	Control	**Fructose**	**Glucose**	**Sucrose**	
Number	24	24	23	23	**-**
Age, (y)	22.5 ± 2.60	23.46 ± 2.34	21.57 ± 2.11	23.26 ± 2.22	0.012
Weight, (kg)	70.80 ± 7.49	69.98 ± 7.94	71.47 ± 6.79	75.85 ± 7.34	0.337
Height, (m)	1.82 ± 0.06	1.79 ± 0.06	1.80 ± 0.05	1.82 ± 0.08	0.039
BMI, (kg/m^2)	21.39 ± 1.57	21.76 ± 1.48	21.95 ± 1.58	22.9 ± 1.09	0.006

Tab 1: Comparison of subject's characteristics at the screening visit (before the beginning of the trial) for the different interventions fructose (Fructose: 80g fructose/d), glucose (Glucose: 80g glucose/d), sucrose (Sucrose: 80g sucrose/d) and control (Control: abstaining from SSBs). Data are described as mean ± SD. P-values <0.05 were considered being significant.

5.2. Effects of SSBs containing fructose, glucose or sucrose on LDL particle distribution

Comparing LDL particle peak size after the four interventions, there was no significant difference between the 4 groups (**Figure 1**). The amount of large (subclass I) particle LDL decreased significantly after the sucrose containing intervention compared with baseline ($p = 0.039$). Despite no significant increase in small LDL particle amount for none of the interventions, there was a tendency of LDL subclass III to increase after the fructose containing intervention ($p = 0.06$) (**Figure 2**). When, however, the effects of the different interventions were compared at a given time point between groups (not within groups), there was not found any significant difference.

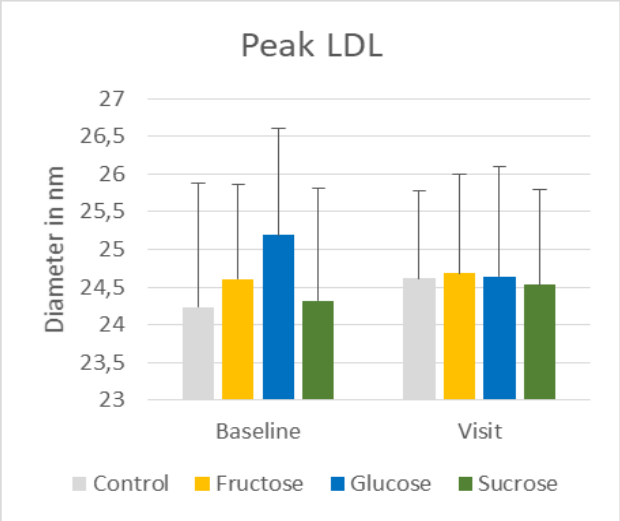

Fig 1: LDL peak diameter after consumption of fructose (Fructose: 80g fructose/d), or glucose (Glucose: 80g glucose/d), or sucrose (Sucrose: 80g sucrose/d) or control (Control: abstaining from SSBs). Baseline stands for the beginning of the trial (week 0) and Visit represents the mean of the three examination days (week 5, 6 and 7).

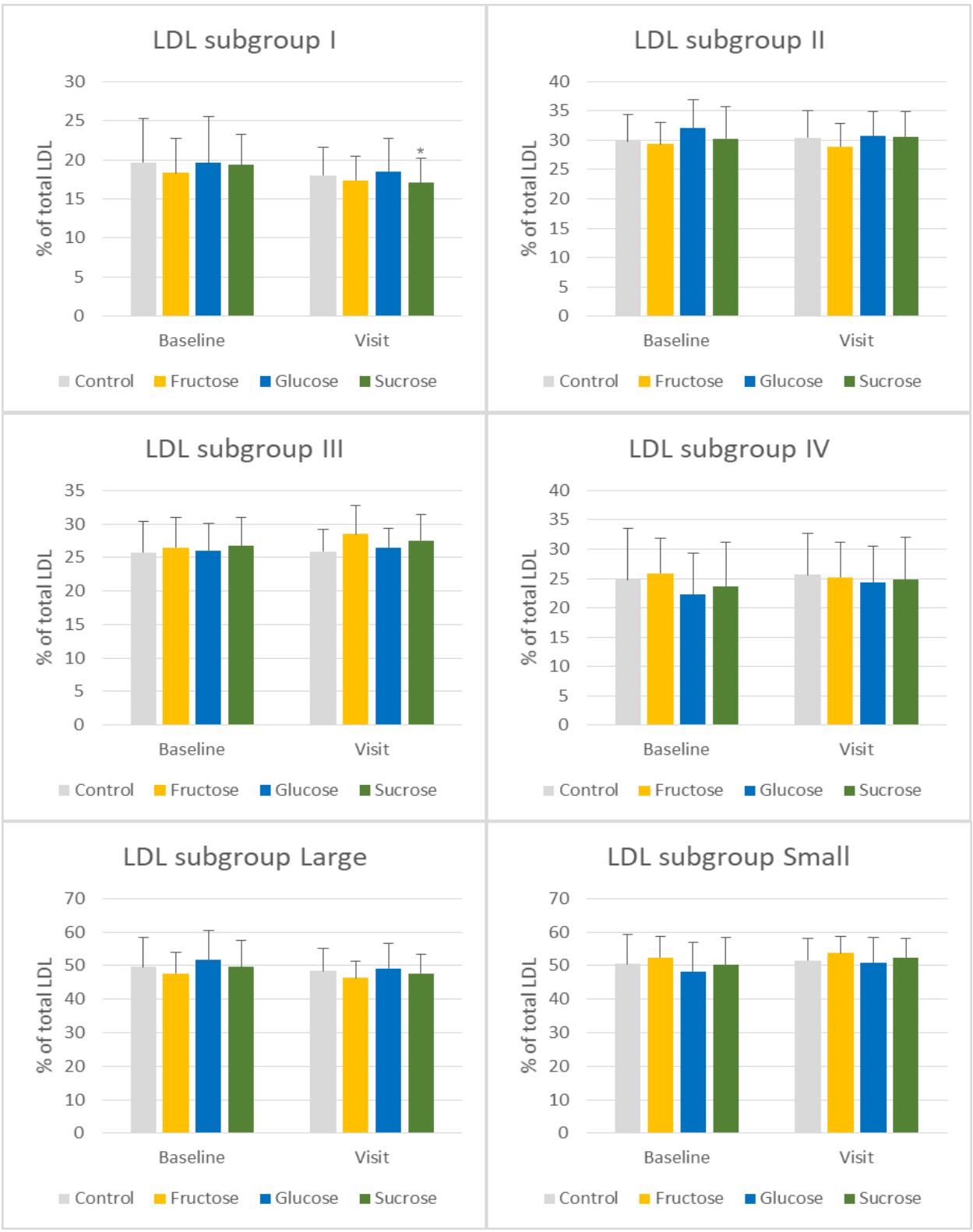

Fig 2: LDL subclasses according to mean percentage of LDL distribution after consumption of fructose (Fructose: 80g fructose/d), or glucose (Glucose: 80g glucose/d), or sucrose (Sucrose: 80g sucrose/d) or control (Control: abstaining from SSBs). LDL subgroup I represents the largest particles (diameter: 27.2-28.5nm), LDL subgroup II the second largest particles (diameter: 25.6-27.2nm), LDL subgroup III the third largest particles (diameter: 24.2-25.6nm), LDL subgroup IV the smallest particles (diameter: 22.0-24.2nm), LDL subgroup Large the LDL subgroup I and II together and LDL subgroup Small the LDL subgroup III and IV together. Baseline stands for the beginning of the trial (week 0) and Visit represents the three examination days together (mean after week 5, 6 and 7). * significantly different from baseline ($p < 0.05$).

5.3. Effects of the SSB intervention in general on LDL particle distribution

When LDL particle peak size was compared between the two interventions (control group versus a combined group of all SSB interventions), there was no significant difference between them (**Figure 3**). The amount of large (subclass I) LDL particles decreased after the SSB intervention significantly ($p = 0.022$), as well as the amount of large LDL particles in general ($p = 0.044$) compared with baseline. There was a significant increase in the amount of small (subclass III) LDL particles ($p = 0.04$) and small LDL particles in general ($p = 0.044$) compared with baseline (**Figure 4**). When, however, the effects of the SSB intervention and the control group were compared at a given time point, there was not found any significant difference.

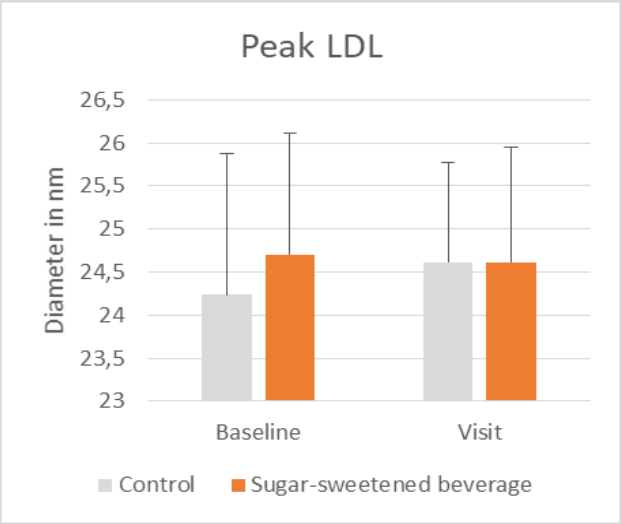

Fig 3: LDL peak diameter after consumption of sugar-sweetened beverages (Sugar-sweetened beverage: 80g fructose/d or 80g glucose/d or 80g sucrose/d) or control (Control: abstaining from SSBs). Baseline stands for the beginning of the trial (week 0) and Visit represents the mean of the three examination days (after week 5, 6 and 7).

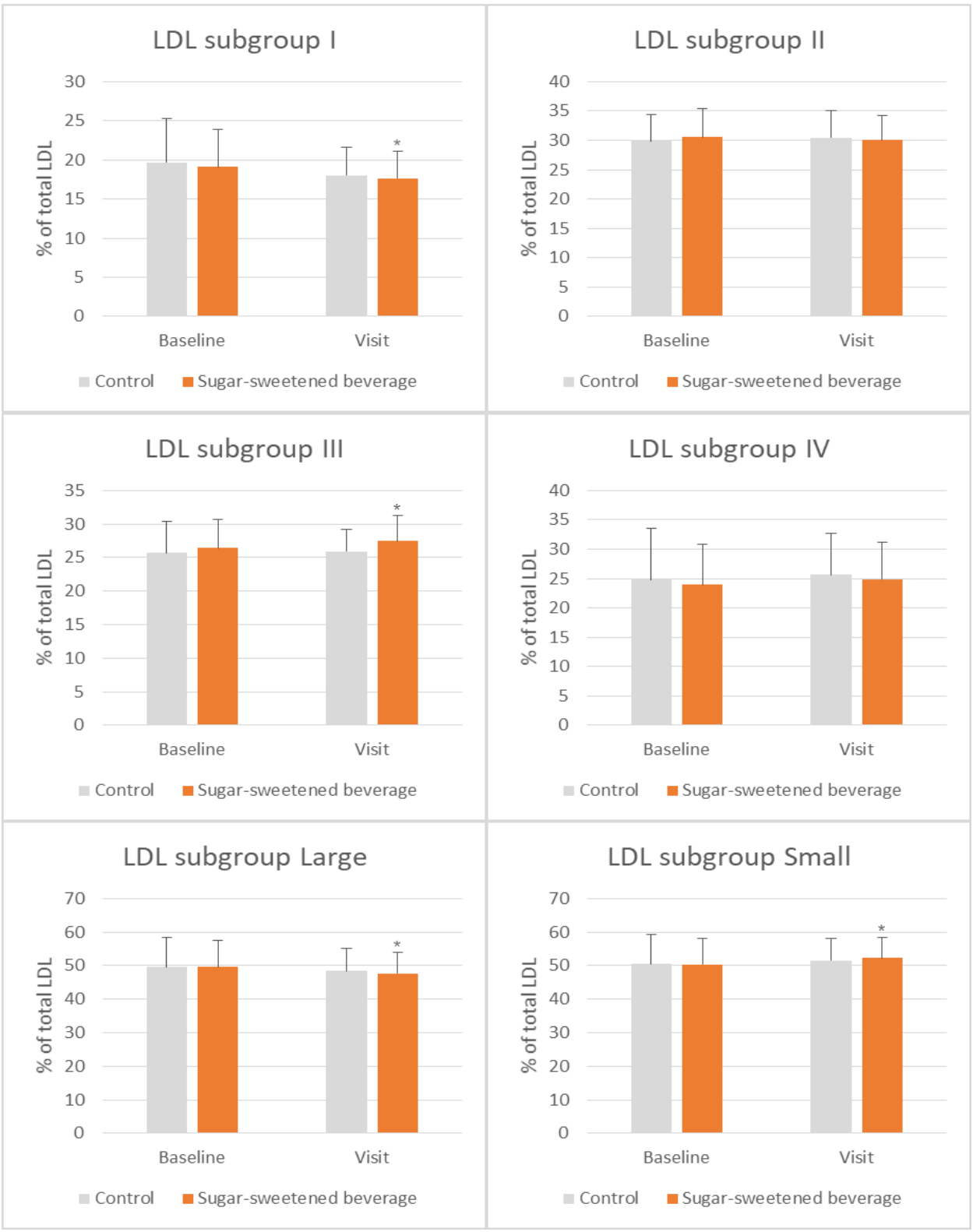

Fig 4: LDL subclasses according to mean percentage of LDL distribution after consumption of sugar-sweetened beverages (Sugar-sweetened beverage: 80g fructose/d or 80g glucose/d or 80g sucrose/d) or control (Control: abstaining from SSBs). LDL subgroup I represents the largest particles (diameter: 27.2-28.5nm), LDL subgroup II the second largest particles (diameter: 25.6-27.2nm), LDL subgroup III the second smallest particles (diameter: 24.2-25.6nm), LDL subgroup IV the smallest particles (diameter: 22.0-24.2nm), LDL subgroup Large the LDL subgroup I and II together and LDL subgroup Small the LDL subgroup III and IV together. Baseline stands for the beginning of the trial (week 0) and Visit represents the mean of the three examination days (week 5, 6 and 7). * significantly different from baseline ($p < 0.05$).

6. Discussion

6.1. Results

In the present study, the amount of small LDL particles increased and the amount of large LDL particles decreased after the SSB interventions. Comparing the effects of SSBs sweetened either with 80g fructose, 80g glucose or 80g sucrose, there was only found a significant decrease in the large, buoyant LDL particles I after the intervention containing sucrose compared with baseline. However, despite having no significant increase in the amount of small LDL particles for none of the interventions, there was a tendency of the small LDL subclass III to increase after the fructose containing intervention. If we summon all SSB interventions and look at it as an SSB intervention in general, there was found a significant decrease in the amount of large, buoyant LDL particles I and generally in the amount of large LDL particles. Moreover, there was a significant increase in the amount of particles in LDL subclass III as well as in small LDL particles after the SSB intervention in general. Of interest, the baseline characteristics of the intervention groups differed significantly in age, height and BMI at the beginning of the trial despite randomization.

6.2. Comparison to other publications

The findings of this study, namely SSBs decreasing LDL particle size in particular after sucrose and fructose-sweetened beverages, but less by glucose-sweetened beverages, are in concordance with the findings of other studies. Aeberli et al. (26). providing healthy young male subjects with the same amount of sugars in form of SSBs daily, found the large, buoyant LDL subclass I to be significantly decreased compared with baseline after consuming fructose and sucrose-sweetened beverages. Moreover, they found fructose and sucrose-sweetened beverages to decrease the large, buoyant LDL subclass I significantly more than glucose-sweetened beverages. The reason why there was no significant effect of fructose decreasing LDL particle size and no significant difference in LDL particle size after the different SSB interventions in the present study may be due to an under-powering of the study in the first place. The study was originally powered for the primary outcome, not for LDL particle size as secondary outcome. Again, this may have prevented the discovery of significant differences between the interventions.

Meanwhile, a study by Stanhope et al. (15) found an increase in LDL and sdLDL both after consumption of fructose-sweetened beverages, but no increase after glucose-sweetened beverages. However, this study was carried out on overweight subjects and the amounts of sugars being used was higher (25% of daily energy). A dietary analysis by Aeberli et al. (2) of overweight school children showed that fructose intake was inversely associated with LDL particle size.

Besides adiposity, total fructose intake was found to be the only dietary factor being a significant predictor of LDL size. This study, however, was carried out on 6 – 14 year old overweight and normal weight children.

6.3. Strengths und limitations of the study

Being carried out in a randomized, controlled, double-blind manner regarding the SSB intervention, is the main strength of the study. Furthermore, using amounts of sugar reflecting real-life intake (80g per day) in form of SSBs counts also to the main strengths of the study. In contrast, most previous studies used very high doses of sugars providing up to 25 - 60% of total daily energy (22). On the other hand, the sugar composition itself did not reflect the composition of sugar being used to sweeten beverages nowadays, for there was only used pure fructose, glucose or sucrose. The study of Aeberli et al. (26) shows the same strengths as just described.

A main limitation is the under powering of the study for it was originally powered for the primary outcome, not for LDL particle size as secondary outcome. This may have prevented the current study to find more significant outcomes. Even though there was used a randomization procedure to appoint subjects to the different intervention groups, another limitation represents the unequal distribution of baseline characteristics at the beginning of the trial. Theoretically, this may have biased the results, however this is unlikely due to the small differences.

6.4. Unanswered questions

Although this study showed adverse effects of moderate SSB consumption on LDL metabolism over a period of 7 weeks, long term impact on LDL metabolism has not been investigated. Further studies are needed to show these effects. Also, more research on the relationship between LDL size and the occurrence of atherosclerosis, CVD risk and the metabolic syndrome needs to be performed. Even though there have already been association studies investigating this (7, 8), none of them appeared to be an intervention study showing the direct impact of SSB consumption on morbidity and mortality. A study like this, however, would not be ethically justifiable, thus studies have to be restricted to surrogate markers.

6.5. Implications

This study shows that even a moderate consumption of SSBs leads to adverse effects on LDL metabolism. Further, sucrose and fructose-sweetened beverages seem to be more detrimental than glucose-sweetened beverages. The diverse effects on LDL metabolism of the different sugars are most probably due to the metabolism of fructose, which in contrast to the metabolism of glucose, does not stand under the regulatory control of insulin and therefore induces lipid synthesis unhindered (20, 21). Only over a short period of 7 weeks, moderate consumption of SSB may worsen LDL parameters in, healthy young, not predisposed men. As moderate SSB consumption leads to an impaired LDL profile, it appears to be a risk factor for metabolic disorders like atherosclerosis and type 2 diabetes (16, 17, 18).

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9. Curriculum vitae

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10. Declaration

Masterarbeit

Ich erkläre ausdrücklich, dass es sich bei der von mir im Rahmen des Studiengangs

Humanmedizin

eingereichten schriftlichen Arbeit mit dem Titel

Moderate sugar-sweetened beverage consumption impairs low density lipoprotein metabolism in healthy young men: A randomized, controlled, double-blinded study

um eine von mir selbst und ohne unerlaubte Beihilfe sowie *in eigenen Worten* verfasste Masterarbeit* handelt.

Ich bestätige überdies, dass die Arbeit als Ganzes oder in Teilen weder bereits einmal zur Abgeltung anderer Studienleistungen an der Universität Zürich oder an einer anderen Universität oder Ausbildungseinrichtung eingereicht worden ist.

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Sanktionen

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Ich bestätige mit meiner Unterschrift die Richtigkeit dieser Angaben.

Datum: 16.09.2017

Name: Liechti Vorname: Marc Raphael

Unterschrift:……..

* Falls die Masterarbeit eine Publikation enthält, bei der ich Erst- oder Koautor/-in bin, wird meine eigene Arbeitsleistung im Begleittext detailliert und strukturiert beschrieben.