



Aus dem Institut für Humanernährung und Lebensmittelkunde  
der Christian-Albrechts-Universität zu Kiel

# **Impact of glycemia and energy balance on metabolic risk in healthy subjects**

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*„Wenn die Neugier sich auf ernsthafte Dinge richtet, dann nennt man sie Wissensdrang.“*

Marie von Ebner-Eschenbach

Für meine Eltern Marita und Ullrich

### **Vorbemerkung**

Die vorliegende Dissertation wurde in kumulativer Form angefertigt und beruht auf einem veröffentlichten sowie einem zur Veröffentlichung eingereichten Manuskript. Zusätzlich zur fortlaufenden Seitennummerierung der Arbeit sind die Seitenzahlen des publizierten Manuskriptes abgedruckt. Das Inhaltsverzeichnis bezieht sich auf die fortlaufende Seitennummerierung der Arbeit.

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## ***Abbreviations***

ADP	Air Displacement Plethysmography
AGE	advanced glycation end product
AMP	adenine monophosphate
ATP	adenine triphosphate
AUC	area under the curve
BMI	body mass index
CGM	continuous glucose monitoring
CHO	carbohydrate
CR	caloric restriction
CVD	cardiovascular disease
DAG	diacylglyceride
DDG	Deutsche Diabetes Gesellschaft
EB	energy balance
EE	energy expenditure
EF	energy flux
EI	energy intake
EPIC	European Prospective Investigation into Cancer and Nutrition Study
ET	energy turnover
FMI	fat mass index
FR	flow rate
Frc	fructose
GFAT	glutamine fructose-6-phosphate amidotransferase
GI	glycemic index
GL	glycemic load
Glc	glucose
GLP-1	glucagon-like peptide 1
GLUT4	glucose transporter 4
HFCS	high-fructose corn syrup
HOMA-IR	Homeostatic Model Assessment-insulin resistance

iAUC	incremental area under the curve
ISF	interstitial fluid
LDL	low density lipoprotein
MAGE	mean amplitude of glycemic excursions
N	nitrogen
O	oxygen
OF	overfeeding
OGTT	oral glucose tolerance test
OJ	orange juice
P	phosphate
PAL	physical activity level
PKC	protein kinase C
REE	resting energy expenditure
ROS	reactive oxygen species
SD	standard deviation
SGLT-1	sodium glucose transporter 1
SSB	sugar-sweetened beverage
TCA	the citric acid cycle
TEE	total energy expenditure
UDP-GlcNAc	uridine diphosphate n-acetylhexoamine
VO <sub>2</sub>	maximal oxygen uptake
WHO	World Health Organisation
wk	week



***Chapter I:  
Introduction***

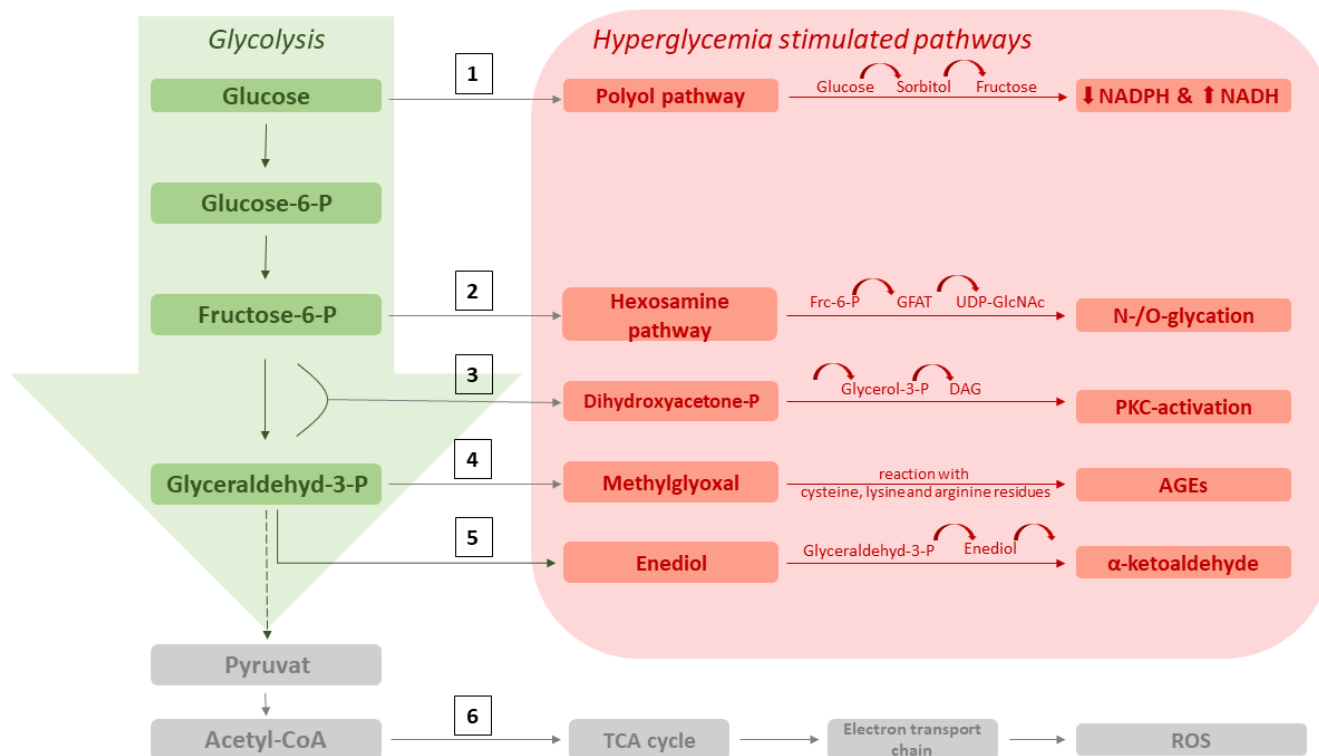
Nutrition is increasingly focusing on blood glucose regulation, because increased blood glucose levels even below the diabetic threshold represent an independent risk factor for the development of cardiovascular diseases (CVD) and constitute a global epidemic problem (Ceriello, 2004; Coutinho et al., 1999; Levitan et al., 2004). In the 1980s, the glycemic index (GI) was developed as a concept for characterizing different carbohydrates in the context of their glycemic response (D. J. Jenkins et al., 1981). The GI provides a standardized comparison of the glucose response of a fixed amount of carbohydrate from a test food to the same amount of carbohydrate from a standard food consumed by the same subject (D. J. A. Jenkins et al., 2002). Since different foods challenge blood glucose levels differently, the selection of low-glycemic foods is important to improve glycemic control (Rizkalla et al., 2004). An extension of the GI-concept is the glycemic load (GL), which describes the product of the quality of carbohydrate-containing food (GI) and quantity (weight) of consumed carbohydrates (Livesey et al., 2013). There is a dose-response relationship, i.e. the higher the glycemic load, the higher the risk of diabetes. The relative risk of developing type 2 diabetes has been shown to increase from a glycemic load > 95 g / 2000 kcal and is 1.45 for each additional GL of 100 g (95% CI [confidence interval]: 1.31, 1.61)). Both, a higher glycemic index and a higher glycemic load of the diet can lead to an increased risk of diabetes, as shown in a meta-analysis of prospective cohort studies (Bhupathiraju et al., 2014). In addition, a regular consumption of high-glycemic beverages for more than one week (20% of daily energy intake in the form of sugar-containing drinks) led to a greater deterioration in insulin sensitivity compared to low-GI beverages in healthy participants (Kahlhöfer et al., 2016). Consumption of low GI beverages led to lower postprandial insulin levels. This is important because chronic, physiologic hyperinsulinaemia leads to the development of peripheral insulin resistance in healthy individuals (Del Prato et al., 1994).

### *Mechanisms of Glucotoxicity*

Prediabetes, characterized by chronically impaired blood glucose responses, is a significant risk factor for type 2 diabetes, with up to 70% of prediabetics eventually developing the disease (Nathan et al., 2007). Type 2 diabetes is associated with insulin resistance, at least at the onset of the disease (DeFronzo, 1997; Gallwitz et al., 2013). Due to persistent

hyperglycemia,  $\beta$ -cells produce more and more insulin in order to lower glucose levels (Szendroedi et al., 2012). At some point, however,  $\beta$ -cells can no longer cope with the permanently high insulin secretion due to the constant hyperglycemia, and they succumb to their exhaustion, what hence leads to an insulin deficiency and causes type 2 diabetes (Maedler & Donath, 2004; Muoio & Newgard, 2008). At the same time, the body and organs suffer from adverse effects of persisting high levels of blood glucose (Johansen & Birkeland, 2003; Kawahito et al., 2009), which is termed glucotoxicity (Del Prato, 2009). **Figure 1** describes mechanisms of hyperglycemic stress that consists of reductive and oxidative stress (Luo et al., 2016). The underlying mechanism of reductive stress is described by the polyol-pathway (pathway 1), which consists of a redox reaction from glucose via sorbitol to fructose (Brownlee, 2005). Hereby NADPH is consumed while an excess of the reducing equivalent NADH is being produced, which can impair NAD-dependent enzyme function or deregulate cell signaling pathways (Luo et al., 2016). On the other hand, there are several pathways that lead to oxidative stress triggered by hyperglycemia including the protein kinase C pathway, the hexosamine pathway, the advanced glycation end products (AGE) pathway and the enediol pathway. Via the hexosamine pathway the synthesis of uridine diphosphate n-acetylhexoamine (UDP-GlcNAc) gets promoted. This reaction is a branched pathway of the glycolysis via fructose-6-phosphate and glutamine fructose-6-phosphate amidotransferase (GFAT, pathway 2, (Schleicher & Weigert, 2000)). UDP-GlcNAc then serves as a substrate for N- or O-glycation of numerous proteins (posttranslational modification) and thus affects protein function (Issad & Kuo, 2008). The formation of dihydroxyacetone from fructose-6-phosphate or glyceraldehyde-3-phosphate, to glycerol-3-phosphate and then to diacylglycerol (DAG) leads to the activation of protein kinase C (PKC, pathway 3, (Xia et al., 2008)). PKC subsequently can drive superoxide production and thus cause oxidative damage to macromolecules (Bey et al., 2004). In another glucotoxic pathway, glyceraldehyde-3-phosphate forms methylglyoxal, which can further react with residues of amino acids (cysteine, lysine, arginine) and results in advanced glycation endproducts (AGEs, pathway 4, (Thornalley et al., 1999)). AGEs contribute to glucotoxicity by an activation of NADPH oxidase and thus cause oxidative stress via the production of superoxides (Gao & Mann, 2009). Enediol is a by-product of autoxidation of glyceraldehyde-3-phosphate in glycolysis, which also produces alpha-ketoaldehyde and reactive oxygen

species (ROS) and therefore increases oxidative stress (pathway 5, (Robertson, 2004; Wolff & Dean, 1987)).



**Figure 1: Hyperglycemia induced pathways**, schematic overview according to Luo et al. 2016; abbreviations: AGE, advanced glycation end products; DAG, Diacylglyceride; Frc, fructose; GFAT, glutamine fructose-6-phosphate amidotransferase; Glc, glucose; N, nitrogen; O, oxygen; P, phosphate; PKC, protein kinase C; ROS, reactive oxygen species; TCA, the citric acid cycle; UDP-GlcNAc, uridine diphosphate-N-acetylhexosamine.

All of these molecular mechanisms take place in the hyperglycemic state and contribute to the magnitude of glucotoxicity, whereas during euglycemic state, glucose is degraded via glycolysis and further on the citrate cycle (pathway 6, (Guo et al., 2012)). Controversial results of an in-vitro study in a  $\beta$ -cell line suggest that glucotoxicity is a continuous process rather than a development depending on a certain threshold of glucose concentration (Gleason et al., 2000). Therefore,  $\beta$ -cells had been cultured in media with different concentrations of glucose. In addition, the impairment of insulin secretion in  $\beta$ -cells developed only after a chronic exposure to high levels of glucose and not immediately and full recovery of cell function was shown to be more likely the shorter the period of antecedent high levels of glucose was.

*Impact of sugar-sweetened beverages on metabolic risk*

Foods with a particular high GI include sugary drinks (Malik & Hu, 2012). Consumption of sugar in the liquid form leads to a rapid gastric emptying and thus results in flooding the gastrointestinal tract and a fast and heavy increase in blood glucose. The following rapid decline in glycemia leads to a high variability of blood glucose levels. Oscillating glucose levels hereby can have more deleterious effects on oxidative stress than sustained chronic hyperglycemia (Monnier et al., 2008).

The consumption of a single can of sugar-sweetened beverage (SSB) already contains the upper limit of the 25-50 grams of free sugar per day, which is recommended by the WHO guideline (World Health Organization, 2015). SSBs include the full spectrum of soft drinks, fruit drinks, juices, energy and vitamin water drinks and contain caloric sweeteners such as sucrose, glucose or high-fructose corn syrup (HFCS), all of which have similar metabolic effects (Hu & Malik, 2010). Casual intake of SSBs and thus an intake of relatively large amounts of energy should be regarded critically, because of an incomplete compensatory reduction in energy intake and a low saturation of SSBs (for review see (Dennis et al., 2009)). Overweight is a major risk factor for the development of diabetes (Colditz et al., 1990; Colditz et al., 1995; Hu et al., 2001). In the United States and United Kingdom, habitual consumption of SSBs was however associated with a greater incidence of type 2 diabetes, even independent of adiposity (Imamura et al., 2015). Likewise, results from *the European Prospective Investigation into Cancer and Nutrition Study* (EPIC) have shown, that daily consumption of 336 g of sugar-sweetened beverages (SSBs) was associated with a 18 % higher risk for type 2 diabetes independent of energy intake and BMI (InterAct Consortium et al., 2013). A fast increase in blood sugar level after consumption of SSBs leads to an increased secretion of insulin and promotes the development of insulin resistance and thus type 2 diabetes especially at a high consumption of sugar-containing soft drinks (O'Connor et al., 2015). In addition, larger fluctuations in blood glucose levels, which occur with SSBs, can damage the  $\beta$ -cells in the pancreas in long-term course (Standl et al., 2011). According to the recommendation of the German Society for Nutrition, the fluid requirement therefore should be covered mainly by water and other low-energy drinks (Deutsche Gesellschaft für Ernährung, 2019).

Epidemiological studies also reveal an association between the regular consumption of sugary drinks, including fruit juice, and the risk of metabolic diseases like an increased risk of gout (Choi & Curhan, 2008; Choi et al., 2010). This increased risk of gout is assumed to be explained predominantly by the consumption of large amounts of fructose, through beverages like lemonades and juices (Choi et al., 2005; Nakagawa et al., 2006). Intake of fructose is known to correlate with elevated serum uric acid levels (Roddy & Doherty, 2010). The fructose-induced purine degradation is considered as a possible mechanism, where adenine triphosphate (ATP) is increasingly being converted to adenine monophosphate (AMP), a precursor of uric acid (Choi et al., 2010). Hyperuricemia is characterized by high levels of uric acid and has been considered as an important risk factor not only for gout but also for obesity, hypertension or coronary heart disease (Abbott et al., 1988; Cannon et al., 1966; Strazzullo & Puig, 2007; Takahashi et al., 1997).

Despite many valuable ingredients like vitamin C, carotenoids, and flavonoids in orange juice (Aschoff, Kaufmann, et al., 2015; Aschoff, Rolke, et al., 2015), most studies do not distinguish between effects of sodas and fruit juices. Orange juice with an annual per capita consumption of 7.8 liters is among the most popular fruit juices in Germany (Sennewald & Heitlinger, 2015). The nutritional evaluation of orange juice is, however, controversial. The health benefits of orange juice are questioned due to its naturally high sugar content and equated with sodas like cola drinks (Ng et al., 2012; WHO, 2003). Despite the high sugar content, the consumption of 100 % orange juice was associated with positive effects on health including a lower total cholesterol and LDL level or a decreased risk for obesity (O'Neil et al., 2012). Anyway, previous studies have shown, that there are components in orange juice affecting the metabolism in a positive (vitamin C, carotenoids and flavonoids) and negative (fructose) way. To what extent and whether the combination of these components impact glucose metabolism and serum uric acid levels remains unclear.

Besides the influence of different foods on glycemia and the consequential metabolic risk, there is a genetic predisposition, which can interact with environmental factors, resulting in the development of type 2 diabetes (for review see (Barroso, 2005)). The field of nutrigenomics tries to understand the complex interaction between nutrition and the human genome in order to develop a diet that is tailored to a person's unique genetic profile with the objective for preventing overweight, type 2 diabetes and cardiovascular diseases (Celis-Morales et al., 2016). Likewise, a whole genome sequencing study enrolling 4 U.S.

communities identified functional sodium glucose transporters (SGLT1) gene variants (Seidelmann et al., 2018). Their results showed, that selective inhibition of SGLT1 protects from diet-induced hyperglycemia in multiple populations. Further investigation of different ways of selective inhibition of this gene variant could provide a strategy for personalized nutrition based on genetic constitution. Apart from the genetic predisposition, interindividual differences in gut microbiota and physical activity contribute to interindividual variance in post-meal blood glucose response of people eating identical meals (Zeevi et al., 2015). A validation of this algorithm in a 100-person cohort was performed and a final randomized controlled intervention revealed significantly lower postprandial glycaemic responses resulting from the personally tailored dietary intervention. One approach for individual improvement of glycemia is therefore the concept of personalized nutrition, with a diet created for every single person based on an algorithm under consideration of dietary habits, gut microbiota and physical activity.

#### *Impact of physical activity on glycemia independent from changes in energy balance*

The development of obesity and type 2 diabetes is largely related to a low physical activity and a positive energy balance. A sedentary lifestyle seems to interfere with the optimal relationship between energy expenditure and energy intake and leads to weight gain, because the low energy expenditure does not induce an equivalent reduction of energy intake (Stubbs et al., 2004; Westerterp, 2010). Hence, a positive energy balance can rapidly impair insulin resistance (Wang et al., 2001), while a negative energy balance, achieved either by increasing energy expenditure through physical activity or by a reduction of the energy intake, results in an improvement of glycemia (Larsen et al., 1997).

Physical activity can improve glycemia on the one hand on an acute basis, by immediately increasing non-insulin-mediated uptake of glucose from the blood into cells. This acute effect is induced by the translocation of glucose transporter 4 (GLUT4) from an intracellular location to the plasma membrane (Goodyear & Kahn, 1998; Hussey et al., 2012; Ploug et al., 1984; Richter et al., 1985; Rose & Richter, 2005). On the other hand, chronic physical activity improves mitochondrial function, increases mitochondrial biogenesis and the expression of glucose transporter proteins in the long-term (Meex et al., 2010; Richter & Hargreaves, 2013). What duration and intensity of physical activity is required to cause a

positive impact on glucose metabolism is controversial (for review see (Bird & Hawley, 2017)).

Apart from the intensity, timing of physical activity in relation to the meal may be important, because of the circadian rhythm in glucose metabolism (Colberg et al., 2009; Gaudet-Savard et al., 2007; Poirier et al., 2000; Terada et al., 2016; Van Cauter et al., 1997). Since energy balance impacts glycemic control, the model of energy turnover (ET) is necessary to examine the effect of physical activity on glucose metabolism independent of energy balance. Energy turnover is defined as the level of energy balance, i.e. a high ET is achieved at a high level of energy expenditure (very physically active) and a corresponding high energy intake. On the contrary, a sedentary behavior with a corresponding low energy intake represents a low ET.



### *Objectives*

This thesis investigates the impact of consumption of sugar-containing beverages on metabolic risk (uric acid, basal and postprandial glycemia) as well as the role of energy turnover on glucose homeostasis among healthy euglycemic subjects.

The following objectives were addressed:

- I. The first objective was to compare the metabolic effects of regular consumption of cola versus orange juice in healthy young adults under free living conditions (Chapter II).

We hypothesized (i) that high sugar intake from cola would increase uric acid levels, in contrast to orange juice consumption and (ii) that cola consumption would result in higher daylong glycemia and impaired insulin sensitivity when compared with orange juice.

- II. The second objective was to investigate the impact of different levels of energy turnover on basal and postprandial glucose metabolism during controlled energy balance (Chapter III).

We hypothesized that low intensity physical activity (performed after the meals) improves (i) postprandial and (ii) 24-hour glucose metabolism independent from energy balance.

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***Chapter II:***

***High intake of orange juice and cola differently affects  
metabolic risk in healthy subjects***





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Original article

## High intake of orange juice and cola differently affects metabolic risk in healthy subjects



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### SUMMARY

**Background:** Higher consumption of sugar-containing beverages has been associated with an elevated risk of type 2 diabetes and gout. Whether this equally applies to cola with an unhealthy image and orange juice (OJ) having a healthy image remains unknown.

**Methods:** In order to investigate whether OJ and cola differently affect metabolic risk 26 healthy adults (24.7 ± 3.2 y; BMI 23.2 ± 3.3 kg/m<sup>2</sup>) participated in a 2 × 2-wk intervention and consumed either OJ or caffeine-free cola (20% Ereq as sugar from beverages) in-between 3 meals/d at *ad libitum* energy intake. Glycemic control, uric acid metabolism and gut microbiota were assessed as outcome parameters.

**Results:** Fecal microbiota, body weight, basal and OGTT-derived insulin sensitivity remained unchanged in both intervention periods. Levels of uric acid were normal at baseline and did not change with 2-wk cola consumption (−0.03 ± 0.67 mg/dL; *p* > 0.05), whereas they decreased with OJ intervention (−0.43 ± 0.56 mg/dL; *p* < 0.01) due to increased uric acid excretion (+130.2 ± 130.0 mg/d; *p* < 0.001). Compared to OJ, consumption of cola led to a higher daylong glycemia (ΔiAUC: 36.9 ± 83.2; *p* < 0.05), an increase in glucose variability (ΔMAGE-Index: 0.29 ± 0.44; *p* < 0.05), and a lower 24 h-insulin secretion (ΔC-peptide excretion: −31.76 ± 38.61 μg/d; *p* < 0.001), which may be explained by a decrease in serum potassium levels (−0.11 ± 0.24 mmol/L; *p* < 0.05).

**Conclusion:** Despite its sugar content, regular consumption of large amounts of OJ do not increase the risk of gout but may even contribute to lower uric acid levels. The etiology of impaired insulin secretion with cola consumption needs to be further investigated.

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### 1. Introduction

Sugar sweetened beverages (SSB) provide the primary source of added sugars in Western diets being most popular in adolescents and young adults in Germany [1–3]. Epidemiological evidence suggests that sugar-containing beverages contribute to type 2 diabetes [4–6] and gout [7–10] by mechanisms that are partly

independent of BMI or weight gain. As a proposed underlying mechanisms, a high dietary glycemic load may contribute to inflammation, insulin resistance, and impaired β-cell function [11–13]. On the other hand, fructose despite having a low-glycemic index, was shown to enhance degradation of purine nucleotides [14] and increase purine synthesis [15,16].

Sugar-containing beverages comprise a great spectrum of soft drinks, fruit drinks, sports drinks, energy and vitamin water drinks and squashes [6]. However, evidence for an impact of different types of beverages on metabolic risk remains limited and inconclusive. Although the perceived health image of orange juice is superior to that of cola, both beverages contain similar amounts of sugars. Daily consumption of one serving of SSB has been

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associated with around 18–20% higher prospective type 2 diabetes risk independent of BMI, whereas this association was found to be insignificant for fruit juices [17,18]. In addition, an increased risk of gestational diabetes has only been reported for cola consumption, whereas other SSBs had no effect [19]. Fruit juice or fructose-rich fruits (e.g. apples and oranges) have been associated with a higher risk of gout [7,20] with one glass of OJ per day increasing the risk by 41% [9], whereas others found no adverse effect of OJ consumption [21]. Associations from epidemiological studies do, however, not necessarily infer causality, and therefore, need to be confirmed by intervention studies. Recently it was recommended by experts that fruit juice shouldn't count to the five a day advice for a healthy fruit and vegetable consumption [22].

Despite their high genuine sugar content, fruit juices are rich in vitamin C that may increase clearance of uric acid [23,24]. In addition, the flavonoid hesperetin in OJ may decrease endogenous uric acid synthesis [25]. Citrus flavonoids such as naringin may exert anti-diabetic effects due to their antioxidant and anti-inflammatory properties [26] and glucose lowering and insulin sensitizing effects [27] e.g. by inhibition of  $\alpha$ -glucosidase or  $\alpha$ -amylase [28]. Lastly, SSBs and fruit juices may differently affect gut microbiota, and thus metabolic risk [29].

The aim of this study was to compare the metabolic effects of high consumption of cola and OJ in healthy young adults. We hypothesized (i) that high sugar intake from cola would increase uric acid levels, in contrast to OJ consumption and (ii) that cola consumption would result in higher daylong glycemia and impaired insulin sensitivity when compared with OJ.

## 2. Methods

### 2.1. Study protocol

The present data are part of a larger quasi-randomized controlled cross-over intervention study that comprised three 2-week intervention periods: OJ consumption with meals, OJ consumption between meals and cola consumption between meals, whereby one cohort started with OJ consumption with meals and the other cohort with between meals and cola consumption represented the last intervention in both rounds [30]. The present evaluation only compared 2-wk cola vs. 2-wk OJ consumption between meals. The study was carried out at the Institute of Nutritional Medicine, University of Hohenheim, Stuttgart, Germany, from April 2016 to December 2016. Participants were recruited at the University of Hohenheim and on social networks, and divided into two cohorts. The first cohort ( $n = 12$ ) was examined between February and March 2016, and the second cohort ( $n = 14$ ) between January and April 2017. Inclusion criteria were: age between 20 and 45 years and a habitual three-meals-per-day structure. Exclusion criteria for enrollment included daily consumption of SSB or fruit juice, regular intake of medication and supplements, chronic disease, smoking, fructose intolerance and special diets (e.g., vegetarian).

All participants had to complete a physical activity interview and a three-day food record before the start of the study. They were requested not to change their physical activity and eating habits throughout the study. During a one week run-in period, consumption of OJ, citrus fruits and SSBs were avoided to facilitate equal baseline conditions. During the 2-wk intervention periods, participants consumed 20% of the individual energy requirements as sugar from orange juice or caffeine-free cola three times a day  $\geq 2$  h after the meals. All participants followed a three-meals-per-day structure without any in-between snacks. Both intervention

periods were separated by one- to two-weeks washout phase. During the intervention periods, consumption of alcohol and additional SSBs and fruit juices was not allowed. For the duration of the entire study, participants were asked to abstain from consumption of citrus fruits and citrus juices.

The present study was designed as a free-living study with 4 obligatory visits at the institute – one at the beginning (day 1,  $T_0$ ) and the end (day 15,  $T_1$ ) of each intervention period. On each of the visiting days, body weight was measured, an oral glucose tolerance test was performed, and blood samples were collected. Weight was assessed with a Tanita scale coupled to the BodPod™ system (COSMED, Rom, Italy). Height was measured with a stadiometer (seca 274, seca, Hamburg, Germany).

The study was registered at clinicaltrials.gov as NCT02974478 and the study protocol was approved by the ethics committee of the Medical Council of Baden-Württemberg, Germany. All participants provided informed written consent before participation.

### 2.2. Intervention with OJ and cola

Beverages used for intervention were 100% OJ with pulp of industrial production, containing 43 kcal/100 mL and 8.8 g sugar/100 mL according to the manufacturer (33% sucrose, 33% glucose and 34% fructose according to own analysis) and a commercial caffeine-free cola containing 44 kcal/100 mL and 11.1 g sugar/100 mL according to the manufacturer (20% sucrose, 41% glucose and 39% fructose according to own analysis). Sugar composition was analyzed according to Pöhl et al. [31] using anion exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD) on an ICS-3000 ion chromatography system (Dionex, Sunnyvale, CA, USA). The OJ contained 185.91 mg/L hesperidin ( $3.4 \pm 0.3$  mg/kg body weight) and 38.87 mg/L narirutin resulting in a mean intake of  $237.6 \pm 41.5$  mg/d hesperidin and  $49.7 \pm 8.7$  mg/d narirutin. On average, OJ has been reported to contain between 200 and 600 mg hesperidin/L and 15–85 mg narirutin/L [32].

The sugar consumed with both beverages was 20% of daily energy requirement. Individual energy requirement was assessed by multiplying resting energy expenditure (REE predicted according to Harris & Benedict [33]) by physical activity level (PAL). Based on their reported daily physical activity individual PAL values ranged between 1.4 (low physical activity) and 1.8 (high physical activity) (estimated according to Brooks et al., 2004 [34]). The study-beverages were weighted in bottles and the filling line was marked. To determine the individual rations, subjects refilled the bottles up to the marking three times a day. Due to a higher sugar content of cola compared to OJ, the amount of liquids required to ensure identical sugar intake was calculated based on their sugar content, thus participants had to drink relatively less cola than OJ. All beverages were provided by the Institute of Nutritional Medicine.

### 2.3. Assessment of fasting and postprandial glucose metabolism

Subjects came to the Institute of Nutritional Medicine between 6:30 and 8:00 am after an overnight fast  $\geq 10$  h. An oral glucose tolerance test (OGTT, intake of 75 g glucose, Accu-Chek® Dextrose O.G.-T., Roche Diagnostics, Mannheim, Germany) was performed. Blood samples were collected before and 30, 60 and 120 min after drinking the glucose-solution. Area under the curve (AUC) was calculated as incremental AUC (iAUC) for 2 h from glucose and insulin OGTT-results using trapezoidal rule [35]. Matsuda Insulin Sensitivity Index was used to evaluate postprandial insulin sensitivity:

$$\text{Matsuda}_{\text{ISI}} = 10000 / \sqrt{(\text{fasting glucose [mg/dL]} \times \text{fasting insulin } [\mu\text{U/mL}]) \times (\text{mean glucose [mg/dL]} \times \text{mean insulin during OGTT } [\mu\text{U/mL}])}$$

[36]. Homeostatic Model Assessment–insulin resistance was used to calculate fasting insulin sensitivity:  $\text{HOMA}_{\text{IR}} = \text{fasting glucose [mg/dL]} \times \text{fasting insulin } [\mu\text{U/mL}] / 405$  [37].

Interstitial glycemia was measured by continuous glucose monitoring (CGM, Dexcom G4 Platinum, Nintamed, Mainz, Germany) for 7 days during both interventions. A small sensor was placed at the back of the upper arm in the subcutaneous tissue to monitor interstitial glucose concentrations. Sensor readings were recorded in intervals of 5 min. The CGM-device was calibrated twice a day against fasting capillary blood samples. Area under the curve (AUC) was calculated as incremental AUC (iAUC) for 18 h (6:00 am–00:00 am) from 3 to 5 valid daylong CGM-data using trapezoidal rule [35].

Glucose variability was determined by mean amplitude of glycemic excursions (MAGE):

$\sum \lambda / \chi$  with  $\lambda > \gamma$ , where  $\lambda$  is the difference from peak to nadir,  $\chi$  is the number of valid observations, and  $\gamma$  is 1 SD of mean glucose in a 24-h period [38,39] using a published macro [40]. Daylong insulin secretion was assessed by 24-h urinary C-peptide excretion at the end of each intervention.

#### 2.4. Blood sampling and analytical methods

Blood sampling was performed by vein cannula. Plasma glucose was determined using hexokinase method (OSR6121, Beckman Coulter, Brea, CA, USA). Serum insulin (Elecsys® Insulin 06923321990, Roche Cobas e801) and urinary C-peptide excretion were measured by luminescence immunoassay (Elecsys® C-Peptide 06923330990, Roche Cobas e801). Levels of uric acid were measured by photometry in fasting blood samples (OSR6098, Beckman Coulter, Brea, CA, USA). Serum potassium level was determined potentiometrically using a Beckman Coulter instrument (AUH1011-1018, Brea, CA, USA).

#### 2.5. Physical activity

Physical activity was continuously monitored using the triaxial activity monitor (ActivPAL™, PALTechnologies, Glasgow, UK). The device was fixed with waterproof patches on the upper thigh midway between the hip and the knee in the correct orientation according to the manufacturer's instruction and worn during both intervention periods.

#### 2.6. Statistical analyses

Data are expressed as mean  $\pm$ SD. Statistical analyses were conducted using SPSS version 22.0 (SPSS, Chicago, IL, USA). Normal distribution was checked by Kolmogorov–Smirnov-Test.  $\text{HOMA}_{\text{IR}}$  did not meet the criteria of normal distribution. Differences between pre and post intervention ( $T_0$  vs.  $T_1$ ) of both interventions were analyzed by mixed-model ANOVA with repeated measures followed by Bonferroni correction. Differences after both interventions ( $\Delta T_1 - T_0$ -OJ vs.  $\Delta T_1 - T_0$ -cola) were analyzed by paired t-test; Wilcoxon-test was used if data were not distributed normally. Associations between changes in uric acid ( $\Delta T_1 - T_0$ -OJ)

versus baseline uric acid concentration or between serum levels of potassium versus daylong insulin secretion were tested using Pearson's correlation. p values  $< 0.05$  were considered as statistically significant. The box-and-whiskers-plot was used to display the distribution of serum potassium levels by quartiles. The lower and upper limits represent the 25- and 75%-quartile of the observed data. The bar within the boxplot represents the median and the whiskers indicate the range of the data. Whiskers maximal length is only allowed to be 1.5 times the interquartile range, outliers are drawn as a single dot.

#### 2.7. Fecal sample DNA isolation, amplification and sequencing

Stool samples were self-collected by patients at the first and last day (days 1 and 15) of the OJ and cola interventions, respectively and used for DNA extraction by mechanical and enzymatic lysis as described before [41]. Hypervariable region V4 of the bacterial 16S rRNA gene was amplified with Golay-barcoded primers 515F and 806R (Sigma Aldrich, St. Louis, USA) and Phusion High-Fidelity PCR Master Mix (Thermo Scientific, Lithuania) see [Supplementary Table S1](#) for primer sequences. PCRs were as follows: 2 min at 98 °C, followed by 30 cycles of 10 s at 98 °C, 15 s at 52 °C, and 10 s at 72 °C and a final extension of 5 min at 72 °C. Equimolar amounts of the resulting PCR amplicons, as determined with the SequelPrep Normalization Plate (Thermo Scientific, Waltham, USA), were combined, concentrated with the DNA Clean & Concentrator-5 kit (Zymo Research, Irvine, USA), adjusted to 100 ng per 60  $\mu\text{L}$  and prepared for sequencing (Illumina MiSeq Regent Kit v3, 2  $\times$  300 bp) with the TruSeq Nano DNA LT Library Prep Kit (Low sample option, Single Indexing). Sequence data are available from the European Nucleotide Archive (<https://www.ebi.ac.uk/ena>) under study accession number ERP105156.

#### 2.8. Sequence processing and microbiota analysis

Sequence reads were processed with QIIME [42]. Sample-specific barcodes were extracted with `extract_barcode.py`, read pairs merged with `join_paired.ends.py`, followed by demultiplexing with `split_libraries.py` (default parameters, except 'barcode\_type "22"'). Primer sequences and spacers between barcodes and primers were removed with `cutadapt` [43]. Reads were clustered into operational taxonomic units (OTUs) based on a similarity threshold of 95% using `pick_open_reference_otus.py` (QIIME) with default settings.

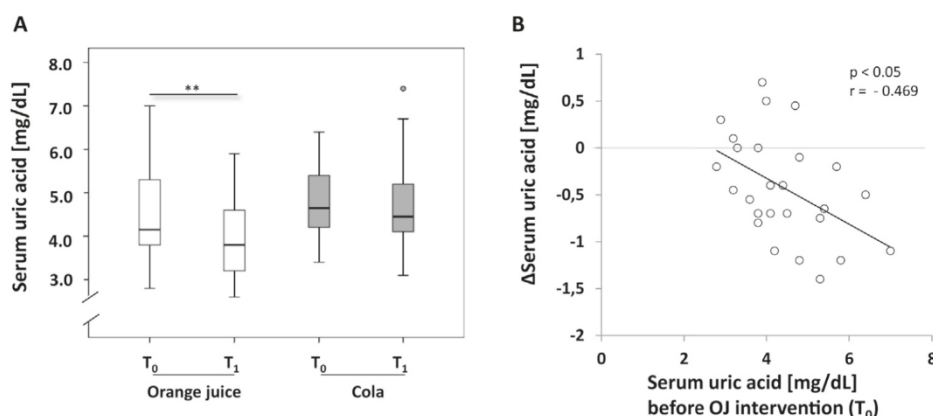
Microbial diversity ( $\alpha$ -diversity), based on the Shannon index, and overall taxonomic microbiota similarities ( $\beta$ -diversity), based on Bray–Curtis Dissimilarity, principal component analysis, analysis of similarities (ANOSIM) and other statistical calculations were calculated and visualized in R ([www.r-project.org](http://www.r-project.org)), using the RStudio environment ([www.rstudio.com](http://www.rstudio.com)). Differentially abundant bacterial taxa were identified with LEfSe [44].

A list of all QIIME and R commands used for sequence processing and analysis is provided as part of the supplement ([Supplementary Table S2](#)).

**Table 1**

Comparison of changes in body weight and glucose metabolism between interventions with orange juice and cola (n = 26).

	Orange juice intervention			Cola intervention			Comparison of changes during both interventions
	T <sub>0</sub>	T <sub>1</sub>	ΔT <sub>1</sub> -T <sub>0</sub>	T <sub>0</sub>	T <sub>1</sub>	ΔT <sub>1</sub> -T <sub>0</sub>	
Body weight [kg]	70.1 ± 14.5	70.3 ± 14.5	0.2 ± 0.9	70.3 ± 14.4	70.1 ± 14.5	-0.2 ± 0.8	p = 0.108
<b>Basal glucose metabolism</b>							
Glucose [mg/dL]	87.4 ± 5.8	88.0 ± 6.0	0.6 ± 6.2	89.7 ± 7.1	87.0 ± 7.3	-2.7 ± 7.0	p = 0.078
Insulin [μU/mL]	7.4 ± 3.6	7.5 ± 2.8	0.1 ± 3.0	7.3 ± 3.8	8.1 ± 3.8	0.9 ± 3.3	p = 0.398
HOMA Index	1.6 ± 0.8	1.6 ± 0.6	0.01 ± 0.67	1.6 ± 0.9	1.8 ± 0.9	0.12 ± 0.77	p = 0.929
<b>OGTT</b>							
Matsuda Index	7.1 ± 2.9	6.2 ± 2.3	-0.9 ± 2.3	7.1 ± 3.4	6.4 ± 2.8	-0.8 ± 2.4	p = 0.703
iAUC <sub>glucose</sub> [mg/dL × 2 h]	39.0 ± 42.8	46.4 ± 30.0	7.4 ± 44.2	46.2 ± 45.2	39.5 ± 37.0	-6.7 ± 37.8	p = 0.171
iAUC <sub>insulin</sub> [μU/mL × 2 h]	81.1 ± 42.0	97.1 ± 48.3	16.0 ± 42.8	93.5 ± 62.1	95.0 ± 40.1	1.4 ± 38.4	p = 0.469

Values are means ± SDs; paired t-test; Wilcoxon test; T<sub>0</sub>, first day of study intervention; T<sub>1</sub>, last day of study intervention.**Fig. 1.** (A) Comparison of serum uric acid levels at the beginning (T<sub>0</sub>) and end (T<sub>1</sub>) of both intervention periods; \*\*p < 0.01; mixed-model ANOVA with repeated measures and Bonferroni correction; (B) relationship between serum uric acid levels before orange juice intervention (T<sub>0</sub>) and changes during orange juice intervention (Δ); \*p < 0.05; r, Pearson's correlation coefficient; (n = 26).

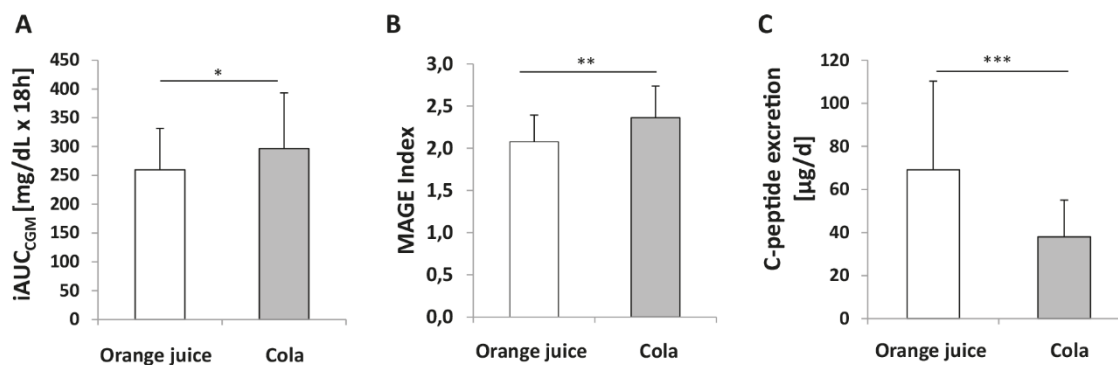
### 3. Results

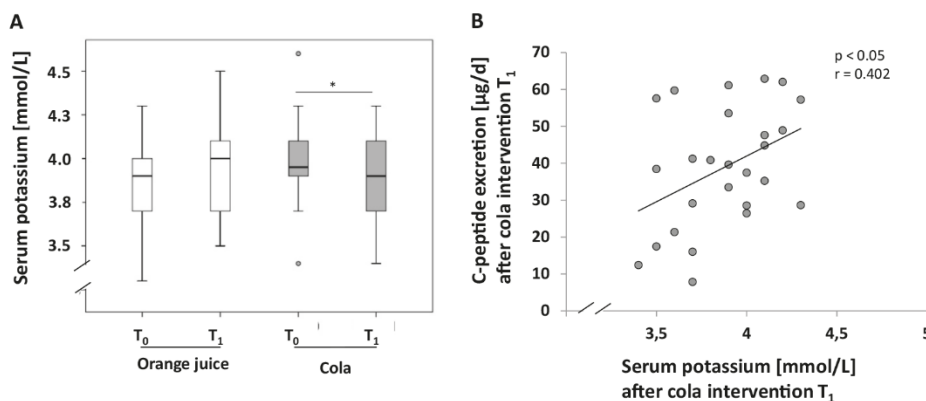
Baseline characteristics of the study population are shown in Table 1. Thirteen women and thirteen men of ages between 20 and 33 years were included. BMI ranged between 19.1 and 33.9 kg/m<sup>2</sup>. According to WHO criteria, 20 subjects were normal weight, five overweight and one obese.

Mean REE and physical activity did not differ between both interventions (OJ vs. cola: 1664 ± 274 and 1666 ± 273 kcal/d; 10,528 ± 3438 and 9917 ± 3871 steps/d; both p > 0.05). The sugar

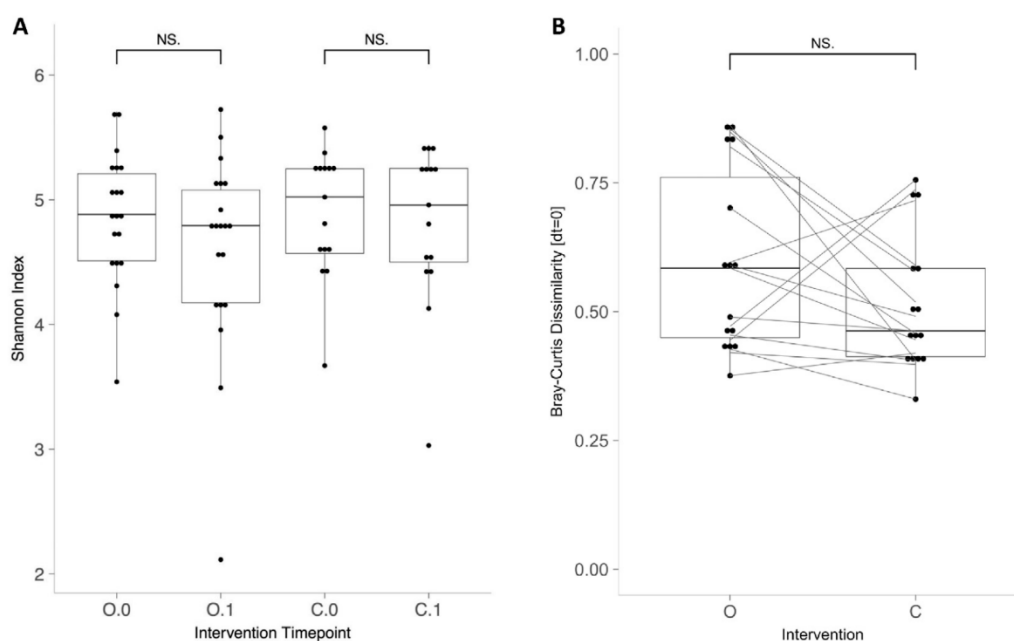
intake with OJ (112.5 ± 19.7 g/d) and cola (113.7 ± 19.7 g/d) was identical by design, whereas the volume and caloric value of consumed beverages was slightly higher in OJ (1278 ± 223 mL/d and 549 ± 96 kcal/d) vs. cola (1024 ± 177 mL/d and 451 ± 78 kcal/d; both p < 0.001). OJ contained 185.9 mg/L of hesperidin and 38.9 mg/L narirutin and 350 mg/L vitamin C (daily intake during orange juice intervention for hesperidin: 237.6 ± 41.5 mg/d, narirutin: 49.7 ± 8.7 mg/d and vitamin C: 447.3 ± 78.2 mg/d).

Body weight as well as parameters of basal and post OGTT glucose metabolism did not change with both intervention periods

**Fig. 2.** Comparison of iAUCs for daylong glycemia (A; average of 3–5 days), MAGE-Index (B; average of 3–5 days) and C-peptide excretion (C; one day at the end of intervention) between orange juice and cola intervention. Values are means ± SDs; \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001; paired t-test; (n = 26).



**Fig. 3.** (A) Comparison of serum potassium levels between the beginning (T<sub>0</sub>) and end (T<sub>1</sub>) of both intervention periods; \*p < 0.05; mixed-model ANOVA with repeated measures and Bonferroni correction; (B) relationship between C-peptide excretion and serum potassium levels after cola intervention; \*p < 0.05; r, Pearson's correlation coefficient; (n = 26).



**Fig. 4.** (A) Microbial diversity of fecal samples collected at the beginning (O.0, C.0) and end (O.1, C.1) of both intervention periods, based on the Shannon diversity index; paired Wilcoxon signed rank test (n = 20 for OJ (O) and n = 15 for cola (C), respectively); NS, not significant; (B) Comparison of changes in taxonomic microbiota compositions between intervention periods, based on Bray–Curtis dissimilarity calculated between the beginning and end of each intervention; paired Wilcoxon signed rank test (n = 15).

(Table 1). Levels of uric acid were all within the normal range at baseline and did not change with 2-wk cola consumption, whereas they decreased with OJ intervention ( $-0.43 \pm 0.56$  mg/dL,  $p < 0.01$ ; Fig. 1A). The decrease in uric acid levels was more pronounced at higher baseline levels ( $r = -0.47$ ;  $p < 0.05$ ; Fig. 1B). Daylong excretion of uric acid increased after two weeks of intervention with OJ ( $\Delta T_1 - T_0$ :  $130.2 \pm 130.0$  mg/d;  $p < 0.001$ ), whereas no change was observed with cola consumption ( $\Delta T_1 - T_0$ :  $-22.7 \pm 214.4$  mg/d;  $p > 0.05$ ).

Daylong glycemia measured by iAUC glucose and glucose variability assessed by MAGE index were higher during cola consumption compared to OJ intervention (Fig. 2A and B). When compared with OJ consumption, 24 h-C-peptide excretion was markedly reduced with cola intervention (OJ:  $70.6 \pm 41.8$  µg/d vs. cola:  $38.8 \pm 16.3$  µg/d;  $p < 0.001$ ; Fig. 2C). Figure 3A shows a decrease in serum potassium after the cola intervention

( $-0.11 \pm 0.24$  mmol/l;  $p < 0.05$ ), whereas potassium levels remained unchanged with OJ consumption ( $+0.11 \pm 0.35$  mmol/l;  $p > 0.05$ ). Lower levels of potassium after cola intervention were associated with lower C-peptide excretion ( $r = 0.402$ ;  $p < 0.05$ ; Fig. 3B).

Neither the OJ nor the cola intervention was associated with significant changes in fecal microbiota diversity (Shannon diversity index;  $p > 0.05$ ; Fig. 4A) or taxonomic composition (Bray–Curtis dissimilarity;  $p > 0.05$ ; Fig. 4B). Analysis of similarity (ANOSIM; [45]) identified significant differences between fecal samples collected from different individuals but not at different time points, i.e. before and after OJ or cola intervention (Supplementary Fig. S1). Bacterial taxa did not show differential relative abundance between the beginning and end points of either the OJ or cola intervention (phylum to genus level; linear discriminant analysis (LDA) effect size < 2). A search for associations between the relative abundances

of bacterial taxa and levels of serum uric acid, C-peptide excretion and serum potassium did not identify significant correlations ( $p > 0.05$ ).

#### 4. Discussion

In line with our hypotheses, two weeks of 20% energy intake as genuine sugar from OJ led to a more favorable metabolic profile compared to the ingestion of same amount of sugar from a caffeine-free cola beverage. Serum uric acid levels significantly decreased following OJ consumption. This result does not support the assumption of an increased risk of gout associated with OJ consumption, as proposed from prospective data of the Health professionals' follow-up study [7] and the Nurses' Health Study [9] using food-frequency questionnaires and the American College of Rheumatology survey criteria for gout. Quite the contrary, our data suggest that regular consumption of larger amounts of OJ may prevent hyperuricemia and gout in healthy individuals. The conflicting results from epidemiological data may be due to confounding: Although the consumption of fruit/vegetable juice has been shown to closely align with a prudent dietary pattern [46], consumption of 100% juice was shown to decline sharply with age [47] and higher age increases the risk of gout [8]. In addition, a lower education level was associated with higher SSB consumption [48] and a lower socioeconomic status may also increase the risk of gout [3,49].

Lower serum uric acid levels after two weeks of OJ consumption as observed in our study could partly be explained by an increased uric acid excretion (see results). The latter may be due to a uricosuric effect of vitamin C. In a randomized controlled trial, serum uric acid levels were inversely correlated with changes in serum ascorbic acid and supplementation with 250–500 mg/d of vitamin C once or for two months was shown to increase glomerular filtration rate, thus reducing serum uric acid levels [23,50]. Similarly, lower levels of uric acid have been observed after four weeks of 500 mL/d OJ consumption [51]. Although in our study the mean intake of vitamin C from OJ was  $447 \pm 78$  mg/d (range 308–580 mg/d), we cannot rule out an additional uric acid lowering effect by a flavonoid-dependent inhibition of xanthine oxidase. Several *in vitro* studies have shown a competitive inhibition of flavonoids such as hesperetin, naringenin and naringin on xanthine oxidoreductase activity [52–54]. In hyperuricemic rats, administration of 5 mg/kg body weight hesperetin partly explained the decrease in uric acid levels observed with OJ [25].

The gut microbiota may play a role in the bioavailability of bioactive flavonoids. Prior to absorption, glycosylated forms of flavanones must be hydrolyzed by the glycosidase activities of the colonic microbiota [55–57]. However, the efficiency of absorption may also be affected by the microbiota because microorganisms can degrade the flavonoid aglycones they produce [58].

Although fructose has immediate uric acid raising effects [14] mediated by an increased ATP degradation to the uric acid precursor AMP, 20% energy intake as sucrose from cola beverages did not raise serum uric acid levels in our healthy subjects. The urate-raising effect of fructose has, however, been shown to be most pronounced in patients suffering from gout and hyperuricemia [14,59–61]. Therefore, prevention of hyperuricemia caused by heavy OJ consumption in healthy adults may not be transferable to treatment, because adverse effects of fructose in these patients may antagonize the urate lowering effects of vitamin C or flavonoids from OJ. Compared to the effect of OJ, pharmacological inhibition of xanthin oxidase by allopurinol and febuxostat can cause severe adverse effects such as hepatitis, nephropathy or allergic reactions [62–64]. Therefore, the efficacy of OJ in the treatment of hyperuricemia in gout patients deserves further study.

A more chronic effect of fructose on uric acid levels may result from fructose-induced hyperinsulinemia [11,65] that may impair renal excretion of urate and correlates with higher serum uric acid levels [66]. Since insulin secretion as assessed by C-peptide excretion was markedly lower during cola intervention, this effect might have impeded an increase in uric acid levels in our study.

The unexpected finding of lower insulin secretion with cola consumption is in line with a higher daylong glycemia and glucose variability compared to OJ consumption (Fig. 2A). Remarkably, prepregnancy consumption of sugar-sweetened cola ( $\geq 5$  vs.  $< 1$  servings/week) was associated with 22% increase in gestational diabetes risk after controlling for potential confounders, whereas no significant association with gestational diabetes was observed for other SSBs [19]. Because the main factor of the pathogenesis of gestational diabetes is a relatively impaired insulin secretion coupled with pregnancy-induced insulin resistance [67], diminished  $\beta$ -cell function by other ingredients of cola beverages may be highly relevant. Caramel coloring in cola-type soft drinks contains advanced glycation end products that may be positively associated with insulin resistance and inflammation [68].

In our study, a slight decrease in serum potassium levels was observed after the intervention with cola (Fig. 3A). This could however be due to higher baseline levels of serum potassium after the OJ intervention. After the intervention with cola, lower daylong insulin secretion (by C-peptide excretion) was associated with lower serum potassium levels (Fig. 3B). Insulin secretion of  $\beta$ -cells is known to be impaired with low serum potassium levels because ATP-dependent K channels are more likely to open and release K ions, which inhibits membrane depolarization and calcium mediated release of secretory granules. Accumulating evidence gives rise to the assumption that a high consumption of cola may lower serum potassium levels [69] and can even cause severe symptoms of hypokalemia in case reports on heavy cola consumption (3–7 l per day; [70–72]). The underlying mechanism remains unclear, and three potential mechanisms of cola-induced hypokalemia have been proposed: (i) increased redistribution of potassium into cells by caffeine [73] or glucose-induced insulin secretion [74]; (ii) increased urinary potassium losses due to caffeine [73] or osmotic diuresis by high sugar intake [74] and (iii) increased potassium losses by osmotic diarrhea caused by the high fructose intake [74]. Since the cola beverage used in our study was non-caffeinated and the subjects had low insulin levels and did not report any incidence of diarrhea upon inquiry of the study team, all explanatory approaches are implausible. The possible link between heavy cola consumption, lower serum potassium levels and insulin secretion may impact recommendations for prevention of gestational diabetes and prevention of type 2 diabetes.

Despite the observed differences in the physiological reactions of study participants to the OJ and cola interventions, no consistent impact of either intervention on the fecal microbiota could be identified across the entire study population. While it is possible that none of these effects were microbiota-dependent, the gut microbiota response to dietary change has also previously been reported to significantly vary between individuals (reviewed by Sonnenburg & Bäckhed [75]) and functional redundancy between taxonomically distinct bacteria from different individuals appears to be a hallmark of the gastrointestinal microbiota [76]. In order to identify and differentiate the effects of OJ and cola consumption on the microbiota, larger study cohorts might therefore be needed with participants stratified based on the initial microbiota compositions.

In conclusion, health consequences of commonly consumed sugar-containing beverages cannot be solely refined to their sugar content because they differ profoundly due to secondary

constituents. In contrast to SSB, orange juice may protect against hyperuricemia.

### Conflict of interest

RC received a donation from the Baumann–Gonser–Stiftung, Bonn, Germany. Research was partly financed by a grant from Baumann–Gonser–Stiftung. FB was awarded a prize by the Baumann–Gonser–Stiftung. Prior to completing the clinical study, one of the authors (JA) has taken up an R&D position at Döhler GmbH (Darmstadt, Germany). Prior to completing the clinical study, one of the authors (RS) has taken up an R&D position at DSM Nutritional Products (Kaiseraugst, Switzerland). None of the other authors reported a conflict of interest related to the study.

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Authors contributions: Writing of the manuscript: ABW, FB, LVD, WFF; data acquisition: FB, FH, LVD, AF, ED, DP, AN, TP, RS; data analysis: FB, FH, LVD, AF; discussion of data and proof-reading of the manuscript: ABW, FB, FH, LVD, AF, ED, DP, WFF, JA, RS, RC; study design: ABW.

### Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.clnu.2018.02.028>.

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**Chapter III:**  
***Impact of energy turnover on the regulation of glucose  
homeostasis in healthy subjects***

The study of this chapter was previously submitted to *Diabetes and Nutrition*.

**Impact of energy turnover on the regulation of glucose homeostasis  
in healthy subjects**

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**Running title:** Energy turnover and glucose homeostasis

**Key words:** energy turnover; non-insulin-mediated glucose uptake; energy balance;  
physical activity

**Abstract**

**Objective:** Sedentary lifestyle increases the risk of type 2 diabetes. The aim of this study was to investigate the impact of different levels of energy turnover (ET; low, medium and high level of physical activity and corresponding energy intake) on glucose metabolism at zero energy balance, caloric restriction and overfeeding.

**Methods:** 16 healthy individuals (13 men, 3 women, 25.1 ±3.9 y, BMI 24.0 ±3.2 kg/m<sup>2</sup>) participated in a randomized cross-over intervention under metabolic ward conditions. Subjects passed 3 x 3 intervention days. Three levels of physical activity (PAL: low 1.3; medium 1.6 and high 1.8 achieved by walking at 4 km/h for 0, 3 x 55 or 3 x 110 min) were compared under 3 levels of energy balance (zero energy balance (EB): 100% of energy requirement (E<sub>req</sub>); caloric restriction, (CR): 75% E<sub>req</sub> and overfeeding (OF): 125% E<sub>req</sub>). Continuous interstitial glucose monitoring, C-peptide excretion and HOMA-IR as well as postprandial glucose and insulin were measured.

**Results:** Daylong glycemia and insulin secretion did not increase with higher ET at all conditions of energy balance (EB, CR and OF) despite a correspondingly higher CHO intake ( $\Delta$  low vs. high ET: +86-135 g CHO/d). At CR, daylong glycemia (p=0.02) and insulin secretion (p=0.04) were even reduced with high compared to low ET. HOMA-IR was impaired with OF and improved with CR, whereas ET had no effect on fasting insulin sensitivity. A higher ET led to lower postprandial glucose and insulin levels under conditions of CR and OF.

**Conclusion:** Low intensity physical activity can significantly improve postprandial glycaemic response of healthy individuals independent of energy balance.

**Introduction**

Higher postprandial glycemia even below the diabetic threshold has been shown to be a risk factor for cardiovascular disease (1,2). In addition, a higher glyceemic load was positively associated with the risk of type 2 diabetes in a meta-analysis of prospective cohort studies (3). Exercise could compensate the negative effects of a high glyceemic load Western diet because it mitigates postprandial glycemia by non-insulin mediated glucose uptake (NIMGU) (4–6). Numerous studies with high intensity exercise showed lower postprandial glucose levels or/and an improved insulin sensitivity in healthy participants (7) as well as in patients with diabetes (8). However, recommendation of high intensity exercise for primary prevention has limitations with respect to injury in high risk groups like untrained obese and elderly subjects. Nygaard et al. investigated the effect of low intensity physical activity on regulation of glycemia in healthy subjects. The authors found a 31.2% decrease of postprandial glucose by a slow 40 min post-meal walk compared to control in healthy women aged > 50 years (9). Manohar et al. confirm this result in 12 healthy control subjects and found a significant reduction of the postprandial glucose area under the curve of 53.1% by walking at a speed of 1.9 km / h (10).

Timing of physical activity may also impact postprandial glycemia because of diurnal variations in energy metabolism and insulin secretion (11). In patients with type 2 diabetes, most (12–16) but not all (17) studies showed that exercise or moderate walking after meals had a greater benefit on postprandial glycemia compared to premeal exercise, whereas premeal exercise had a greater impact on improvement of fat oxidation (18).

A major drawback of all previous studies is the condition of uncontrolled energy intake with physical activity. Because physical activity leads to an increase in energy expenditure and increases energy intake only partially to the corresponding enhanced energy requirement (19) a negative energy balance is likely created that impacts glucose metabolism. Zero energy balance, where energy intake corresponds to energy expenditure, is therefore necessary to investigate the effect of physical activity on regulation of glycemia without the confounder of negative energy balance. A condition of fixed energy balance at a varying physical activity level is defined as energy turnover (ET) or energy flux. A low energy turnover, i.e. a low energy intake with a low energy expenditure, resembles an inactive lifestyle whereas a high energy turnover can be achieved by an increase in physical activity at a correspondingly higher energy intake. A high energy turnover might compensate for

the negative impact of short-term overfeeding on glucose metabolism. Sedentary behavior is known to facilitate a positive energy balance e.g. over the weekend, on holidays, at periods of celebration, or during vacations (20,21). There is evidence that one day of sitting without adjusting for the lower energy expenditure already leads to a reduced insulin action (-39%) similar to changes reported after longer periods of bed rest or large reduction in ambulation (22).

The aim of the study was to investigate the impact of low, medium and high ET obtained by different durations of low intensity physical activity (performed after the meals) on the regulation of basal and circadian glucose metabolism at zero energy balance. The impact of these three levels of energy turnover on glucose metabolism was also examined under randomized conditions of controlled overfeeding (OF) and caloric restriction (CR).

## Methods

Sixteen healthy subjects were recruited via announcements on social networks and at the campuses of the University of Stuttgart and Hohenheim. Inclusion criteria were age between 20 and 40 years and a normal physical activity in daily routine. Exclusion criteria for enrollment included regular intake of medication and supplements, chronic disease, smoking, claustrophobia and special diets or any food intolerances. The study was carried out at the Institute of Nutritional Medicine, University of Hohenheim, Stuttgart, Germany, from December 2016 to March 2018.

### Study design

The randomized controlled cross-over intervention comprised three 1-week interventions: zero energy balance (EB), caloric restriction (CR) -25 energy requirement (Ereq) %, overfeeding (OF) +25 Ereq%. Each week was performed under three different levels of energy turnover: one inactive day (PAL 1.3 = low ET), one day of normal physical activity (PAL 1.6 = medium ET) and one day of high physical activity (PAL 1.8 = high ET; **Figure 1**). The three intervention days per week were separated by a washout day. A subject who completed the entire study thus went through nine different intervention days and additional 3 days, where the energy requirement for low, medium and high ET was measured with *ad libitum* energy intake (baseline week). Each of the nine intervention days consisted of a 36 hour stay in the metabolic chamber. Participants entered the metabolic chamber in the evening before each test day. The measurement period started at 6 am in the morning and ended 24 hours later. The sleeping period was constant and always from 10:30 pm to 6 am. EB was conducted as the second intervention-week, whereas CR and OF were performed in randomized order as the first or third week of study. All intervention days (low, med, high) were kept in randomized order, which was obtained by block randomization.

The study was registered at clinicaltrials.gov as NCT03361566 and the study protocol was approved by the ethics committee of the Medical Council of Baden-Württemberg, Germany. All participants provided informed written consent before participation.

### 24h-energy expenditure

Energy expenditure at different levels of activity (baseline week) was measured in a room calorimeter and was used to determine individual energy requirement (for details see (23)). Total energy expenditure (TEE) was determined at a constant flow rate of 120 l/min fresh

air by continuously measuring rates of oxygen and carbon dioxide concentrations using the Promethion integrated whole room indirect calorimeter system (Sable Systems International, Las Vegas, USA) and the Weir equation (24). Using energy intake and TEE-data, individual energy balance for different ET levels was calculated as  $\Delta$ energy balance [%] =  $(EI / TEE \times 100) - 100$ . The influence of energy turnover on macronutrient oxidation and macronutrient balances is subject of a separate publication (Nas A et al., submitted 2019).

#### Body composition

Height was measured using a stadiometer (seca 274, seca GmbH & Co.KG, Hamburg, Germany). Body weight was measured in the morning before breakfast at the beginning and end of each energy balance condition using a calibrated electronic scale (seca mBCA 515, seca GmbH & Co.KG), in light clothes, without shoes and after voiding. Before starting the study, body composition of the subjects was analyzed using Air Displacement Plethysmography (ADP) by the BodPod™ Body Composition System (COSMED USA, Inc., Concord, CA, USA) as described elsewhere (25).

#### Standardization of the diet

To ensure equal baseline conditions, each intervention was preceded by a 3-day entrance phase with controlled macronutrient composition of the diet. On intervention days, a strict daily structure with three meals was achieved. Breakfast was served at 7 am, lunch at 1 pm and dinner at 7 pm. Participants consumed a western style diet with a macronutrient ratio of 50% carbohydrates, 35% fat and 15% protein, which was kept constant throughout the whole study period. A constant macronutrient composition was achieved by weighing each food item and by evaluation of the menus with the software Prodi®6 (Scientific Publishing Company, Stuttgart). Meal composition on intervention and washout days as well as during the entrance phase was kept constant. During the baseline week for assessment of energy requirement at different levels of ET, as well as in the entrance phase under free living conditions and during the washout days, participants received all food in abundance and leftovers were weighed back to assess energy and macronutrient intake. On all intervention days during EB, CR and OF, subjects were instructed to eat their entire meal within 30 minutes without any leftovers. To ensure a controlled dietary intervention, all food consumed throughout the whole study was provided by the institute. For the duration of the entire

study, participants were asked to abstain from consumption of alcohol and any additional food or snacks except mineral water, herbal and fruit tea that were allowed *ad libitum*. Glycemic load (GL) of a food was calculated by multiplying GI of each food (26) by the amount of CHO in grams provided by the food and dividing the total by 100 (27).

#### Physical activity

Physical activity was recorded using a triaxial activity monitor, activPAL™ (Paltechnologies Ltd., Glasgow, UK). The device was continuously worn on the middle of the thigh fixed with a waterproof patch. ActivPAL™ Professional v7.2.32 software was used for data analysis. The subjects had to pass three different levels of physical activity depending on the energy turnover. All physical activity levels were achieved by walking with a constant speed of 4 km/h on a treadmill (Kettler Track 9, Kettler GmbH, Ense-Parsit, Germany) for different durations. On the day with low ET (PAL 1.3), subjects were sedentary by sitting or lying throughout the day. On the medium ET day with normal activity, subjects walked on the treadmill three times a day 10 min after finishing meals (PAL = 1.6) for 55 min. On the high ET day, walking units were increased to 110 min each (PAL = 1.8). Before the start of the study, it was tested which walking time and walking speed were appropriate to reach the given PALs. Subjects were told to refrain from exercise during the entrance phase and on the washout days and only follow their usual everyday activities. Due to technical problems with the device, there is no valid data of physical activity of one subject for the whole intervention and two participants had missing data for one energy balance condition each.

#### Assessment of glucose metabolism

In the morning of the intervention days and the following mornings, fasting blood samples were taken. Fasting glucose concentration was determined via hexokinase method (OSR6121, Beckman Coulter, Brea, CA, USA). Fasting serum insulin (Elecsys® Insulin 06923321990, Roche Cobas e801) and 24-hours urinary C-peptide excretion were measured by luminescence immunoassay (Elecsys® C-Peptide 06923330990, Roche Cobas e801). Homeostatic Model Assessment-insulin resistance (HOMA-IR= fasting glucose (mg/dl) x fasting insulin (μU/ml) / 405, (28)) was used to calculate fasting insulin sensitivity. Daylong glycemia at different levels of energy turnover, was assessed by continuous interstitial glucose measurement (CGM, Dexcom G4 Platinum, Nintamed, Mainz, Germany) for at least 24 hours during all nine intervention days. A small sensor was



placed at the back of the upper arm in the subcutaneous tissue. Sensor readings were recorded in intervals of 5 min. The CGM-device was calibrated three times a day before meals against fasting capillary blood samples. Incremental area under the curve (AUC) was calculated (iAUC) for 18 h (6:00 am - 12:00 pm) using trapezoidal rule (29), whereby iAUC includes only the positive area under the curve. Glucose variability was described by mean amplitude of glycemic excursions (MAGE-index) and calculated using a published macro (30). Daylong insulin secretion was assessed by 24-h urinary C-peptide excretion.

Results of the continuous glucose monitoring were adjusted for the difference between fasting serum glucose and fasting CGM-glucose ( $\text{Glucose}_{\text{CGM}} - \text{Glucose}_{\text{venous}}$ ) to calculate postprandial iAUC of glucose. Plasma samples for the measurement of postprandial insulin were collected 30, 60 and 120 min after each meal in BD™ P800 tubes (Becton Dickinson Inc., Franklin Lakes, USA) and measured using a Bio-Plex Pro™ human Diabetes 3-Plex Kit (Bio-Rad, Hercules, USA). Data analysis was performed with Bio-Plex Manager™ Software 6.1. iAUC was calculated from post-meal insulin values over 2-hours.

One participant was excluded for the analysis of daylong insulin secretion (n=15) and HOMA-IR of two subjects was missing for low energy turnover at the condition of EB (n=14).

### Statistical analyses

Data are expressed as mean  $\pm$ SD. The statistical software R (2017) was used to evaluate the data. Data evaluation started with the definition of an appropriate statistical mixed model (31,32). The data were assumed to be normally distributed and to be heteroscedastic with respect to different levels of energy balance and ET. These assumptions are based on a graphical residual analysis. The statistical model included energy balance (zero energy balance, caloric restriction, overfeeding) and ET (low, medium, high), PrePost (before intervention, after intervention) as well as their interaction terms as fixed factors. The ID and energy balance, nested in ID, were regarded as random factors. Also, the correlations of values between intervention days as well as the correlations between pre and post measurements were taken into account (auto-correlation). Based on this model, a Pseudo  $R^2$  was calculated (33) and an analysis of variance (ANOVA) was conducted, followed by multiple contrast tests (e.g., see (34,35)) in order to compare the several levels of the influence factors. p values < 0.05 were considered as statistically significant. Box-and-whiskers-plots were used to display the distribution of  $\text{iAUC}_{\text{CGM}}$ , C-peptide-excretion and

HOMA-IR. Based on results of a previous study of our group (36), a total sample size of  $n = 8$  was required to assess these differences in daylong glycemia at a  $\alpha$ -level of 0.05 and a power of 95% (hypothesized effect size = 1.4).

## Results

Three women and thirteen men aged  $25.1 \pm 3.9$  years with a BMI  $24.0 \pm 3.2$  kg/m<sup>2</sup> and a FMI  $5.3 \pm 3.2$  kg/m<sup>2</sup> were included in this study. According to WHO criteria, 10 subjects were normal weight, five overweight and one obese. Regarding body composition, four subjects had a FMI above the age and sex adjusted 95<sup>th</sup> percentile (37).

Body weight remained unchanged during all conditions of energy balance ( $\Delta$ CR:  $0.0 \pm 0.3$  kg;  $\Delta$ EB:  $-0.1 \pm 1.1$  kg;  $\Delta$ OF:  $0.3 \pm 0.3$  kg; all  $p > 0.05$ ). Daily step count and PAL were different by design between the three levels of ET in all conditions of energy balance (all  $p < 0.05$ ; **Table 1**) but did not differ at the same ET between the three energy balance conditions (all  $p > 0.05$ ; except low ET: CR vs. OF,  $p < 0.05$ ). Energy balance was 3.4%, 0.0% and 1.6% during zero EB, -20.5%, -19.5% and -21.0% during CR and 27.6%, 25.4% and 24.2% during OF for low, medium and high ET, respectively (EB vs. CR vs. OF, all  $p < 0.001$ ).

As intended by study design, total caloric intake, as well as absolute carbohydrate-, fat- and protein-intake increased within each energy balance with higher ET (all  $p < 0.001$ ) and differed between conditions of energy balance (all  $p < 0.001$ , **Table 1**). Macronutrient composition was  $49.4 \pm 0.5\%$  CHO,  $34.9 \pm 0.7\%$  fat and  $14.8 \pm 0.1\%$  protein and did not differ throughout the different phases of the study. Total daily glycaemic load ranged between 123.8 – 285.2 g/d (**Table 1**). Daily glycaemic load was distributed with 27.4 % at breakfast, 28.8 % at lunch and 43.8 % at dinner.

### Effect on daylong and basal glucose metabolism

Changes in daylong glycemia and insulin secretion are shown in **Figure 2**. During CR, daylong glycemia and insulin secretion were higher with low compared to high ET ( $p < 0.05$ , **Figure 2**). Despite a higher intake of carbohydrates with increasing level of ET ( $\Delta$  low vs. high ET: +86-135 g CHO/d; **Table 1**), there was no increase in daylong glycemia and insulin secretion with higher ET during EB and OF. During CR, glucose variability (MAGE-Index) was higher with low ET compared to medium and high ET (low ET:  $3.1 \pm 1.0$ , medium ET:  $2.3 \pm 0.5$ , high ET:  $1.9 \pm 0.4$ ; both  $p < 0.05$ ). Fasting insulin sensitivity was improved with CR and impaired with OF (all  $p < 0.01$ ) irrespective of ET (**Figure 3**).

**Effect on postprandial glucose metabolism**

Changes in energy balance did not impact postprandial glucose and insulin responses (EB vs. CR and EB vs. OF; all  $p > 0.05$ ; except postmeal insulin at medium ET CR vs. EB,  $p < 0.05$ ). A higher ET led to lower cumulative postprandial glucose and insulin levels (mean of postprandial iAUCs of breakfast, lunch and dinner) under conditions of CR and OF (low vs. high ET for insulin: -28.9 % and -44.5 % both  $p < 0.001$ , and for glucose -34.2 % ( $p < 0.001$ ) and -29.7 % ( $p = 0.066$ )). Changes in postprandial glucose and insulin response are shown in **Figure 4**, separated for breakfast, lunch and dinner. After breakfast, post-meal glucose ( $p < 0.05$ ) and insulin ( $p < 0.01$ ) levels were lower with high compared to low ET during CR only, during OF only postprandial insulin response was lower with high compared to low ET ( $p < 0.05$ ). After lunch, ET had no effect on postprandial glucose levels whereas postprandial insulin levels decreased with higher ET during all conditions of energy balance (all  $p < 0.05$ ). After dinner, the response in postprandial glucose decreased with increasing ET under all conditions of energy balance (all  $p < 0.01$ ) whereas a decrease in postprandial insulin levels with higher ET was only observed during OF ( $p < 0.001$ ).

## Discussion

The main finding of the study was that higher energy turnover improved postprandial glucose and insulin responses at all levels of energy balance (Figure 4). The improvement of postprandial insulin levels was most pronounced after lunch and for glucose after dinner. Postprandial insulin sensitivity was therefore enhanced by low intensity physical activity especially after lunch and dinner. The lack of a significant change in basal glycemia and HOMA suggests, that energy turnover primarily improves glucose uptake in skeletal muscle. In addition, only healthy normoglycemic subjects were investigated which makes an intervention effect on basal glucose and insulin levels unlikely (Figure 3).

### *Timing and frequency of physical activity*

Physical activity in our study was always performed in a postmeal situation. Colberg et al. described, that an increase in daily activity of low intensity improves blood glucose management and lowers postmeal hyperglycemia, especially when physical activity was performed directly after meals (38). Because peak glucose values usually appear within 90 min postprandial (39), the optimal timing for physical activity has been assumed to be 30 min after meal onset (40). The benefit of a postmeal compared to a premeal bout of physical activity on glycemia might be due to the synergistic effect between insulin and non-insulin-mediated glucose uptake by muscular contraction (41). Elevated blood glucose levels two hours after lunch have been shown to increase the risk for cardiovascular events by 50% and the risk for mortality by 89% in patients with type 2 diabetes (42). Because the exercise-effect is insulin-independent, it works well in patients with diabetes, too (43–45). Therefore postmeal exercise may be an effective way to improve glucose control in patients with type 2 diabetes (46). Borrer et al. recommended that individuals with type 2 diabetes should focus on increasing energy expenditure after the largest meal of the day. However, 15 min of postmeal walking (3 times per day) was more effective in lowering 3 hours postdinner glucose levels than a 45 min walk in the morning or afternoon compared to control day in subjects at risk for impaired glucose tolerance ( $p < 0.01$ ) (16).

In the present study, postmeal glucose and insulin levels decreased most after lunch and dinner (Figure 4). This effect could be due to a cumulative effect of walking intervals over the intervention day or it might be explained by the circadian difference insulin sensitivity. Healthy nondiabetic individuals have a higher insulin sensitivity in the morning and show a

diurnal decrease in insulin sensitivity, which is in part the result of better  $\beta$ -cell responsiveness (11). When exercise was performed after lunch, there was a minimal impact on glycemia over the following 24 hours (47). By contrast, exercise performed in the evening has been shown to reduce glycemia during exercise and the overnight period in patients with type 2 diabetes (13). However, the glucose- and insulin-lowering effect of exercise after a meal does not seem to persist at the following meal suggesting that these improvements are short-lived (12).

In summary, timing and frequency of physical activity seem to play a significant role for improvement of glucose metabolism. With respect to feasibility and preventive recommendations it is important that breaking up prolonged sitting with short bouts of walking or simply standing already has positive effects on glycemia (48–50). The implementation of several smaller walking-units is easier to realize in everyday life, especially in risk groups like older persons.

#### *Intensity and type of physical activity*

In our study, a positive effect on postprandial glycemia was achieved even by low intensity walking on the treadmill at 4 km/h. Glucose uptake is enhanced with higher intensity of physical activity because of increased glucose delivery, transport and metabolism (51). Further studies are needed to clarify a dose-response relationship for light intensity activity thresholds (52). The type of exercise involves different muscle groups and masses and may therefore differently affect the extend of the glucose lowering effect. In line with this presumption, climbing stairs led to a more rapid decrease in postprandial blood glucose levels compared with cycling (53). In patients with type 2 diabetes (54) as well as in healthy volunteers (9,10,55,56), slow post meal walking was able to lower postprandial glucose and insulin levels compared with sedentary controls. We found reduced postprandial insulin levels at high compared to low energy turnover during CR and OF (Figure 4D-F) although the intake of CHO was considerable higher (e.g. during OF at low ET  $366 \pm 52$  g vs.  $507 \pm 85$  g at high ET). This is likely due to improved non-insulin mediated glucose uptake by physical activity.

### *Physical activity and energy balance*

So far, all studies that investigated the impact of low intensity physical activity on postprandial glycemia did not consider energy balance. Hence, the observed postmeal glycemia-lowering effect of physical activity could be also explained by a higher energy expenditure and a resulting caloric deficit. This negative energy balance alone could explain the improvement in glycemia. This assumption is supported by the results of Larsen et al. who observed the same effect on glycemia by 45 min of exercise after breakfast or an equal energy deficit achieved by a lower energy intake at breakfast (12). Both conditions led to a similar decrease in postprandial glycemia after breakfast. However, in the present study caloric restriction (between -19.5% and -21.0% energy balance) did not improve postmeal levels of glucose or insulin compared to zero energy balance. Similarly, overfeeding (between +24.2% and +27.6% energy balance) did not worsen postprandial glucose metabolism (Figure 4). On the other hand, a positive energy balance can result from inactivity (57) and thus might explain the deterioration in insulin sensitivity (22).

Postprandial glucose ( $p=0.066$ ) and insulin levels ( $p<0.001$ ) were also improved by a high ET compared to a low ET during overfeeding (Figure 4). The positive effect of physical activity on postprandial glucose metabolism was therefore independent of energy balance. Similar to our findings, 25% overfeeding combined with a single bout of exercise (~ 60 minutes of ergometer or treadmill at 60% of  $VO_2$  peak) also led to a 20% decrease in postprandial insulin AUC ( $p<0.05$ ), compared to overfeeding alone (58). Impaired glucose metabolism by overfeeding is well established (59) and may not have been detectable in our study because of the short duration and relatively low energy surplus.

### *Strength and limitations of the study*

A strength of our study was the randomized cross-over study design that strictly controlled for possible confounding factors such as energy balance, diet, exercise and sleeping behavior. The use of healthy participants may limit the generalizability of the results to people with obesity or type 2 diabetes. Studies that did not control energy balance already provided some evidence that postprandial exercise is also effective to improve glucose control in patients with type 2 diabetes (43–46,54).

Since our study only covers a 24-hour intervention, the results are not transferable to long-term effects of physical activity. However, short-term changes in eating and motion

behavior, are very common and realistic in everyday life (20,21). In addition, the quite long walking duration on the treadmill during high ET (330 min) could appear unrealistic, but the stay in a metabolic chamber is an artificial setting that prevents other spontaneous physical activity of daily life.

*Conclusion*

Low intensity postprandial physical activity is effective to lower postmeal glucose and insulin levels in healthy adults independent of energy balance. Since higher postprandial glycemia even below the diabetic threshold has been shown to be a risk factor for cardiovascular disease (1,2), walking after the meals is an advisable preventive strategy.



### **Acknowledgements**

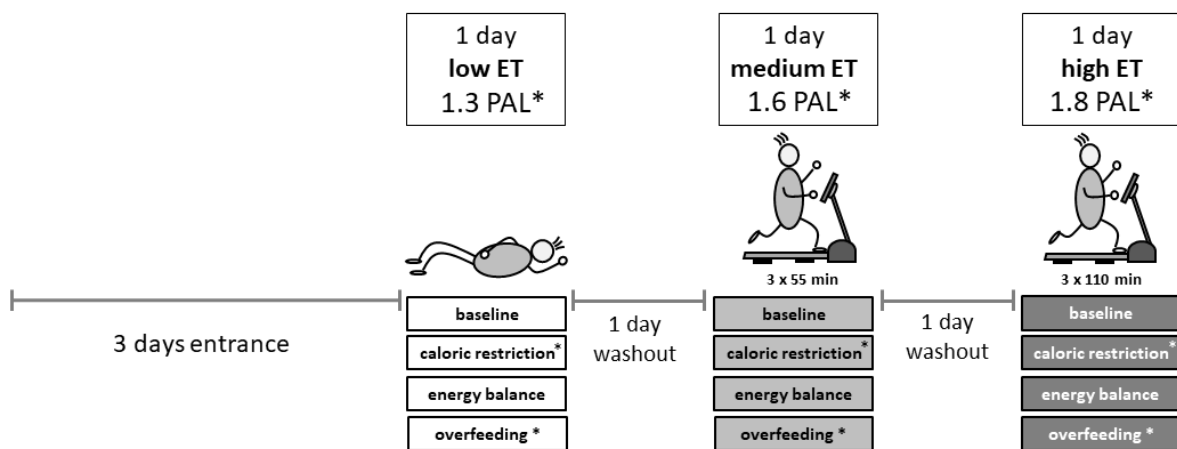
**Authors contributions:** Writing of the manuscript: FB, ABW; data acquisition: FB, FAH, AN; data analysis: FB; MH provided statistical support; discussion of data: FB, MJM, ABW; proof-reading of the manuscript: FB, FAH, AN, MJM, ABW; study design: ABW

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### **Conflict of interest statement:**

The authors declare that there is no conflict of interest.

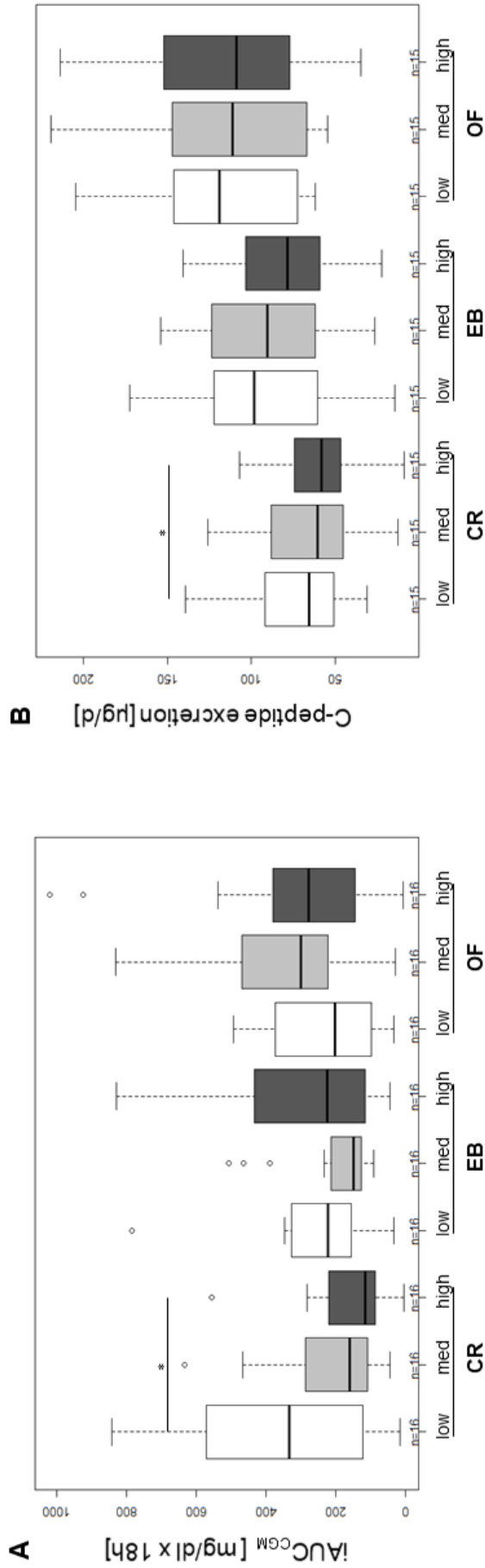


**FIGURE 1: Schematic study protocol;** \* randomized order.

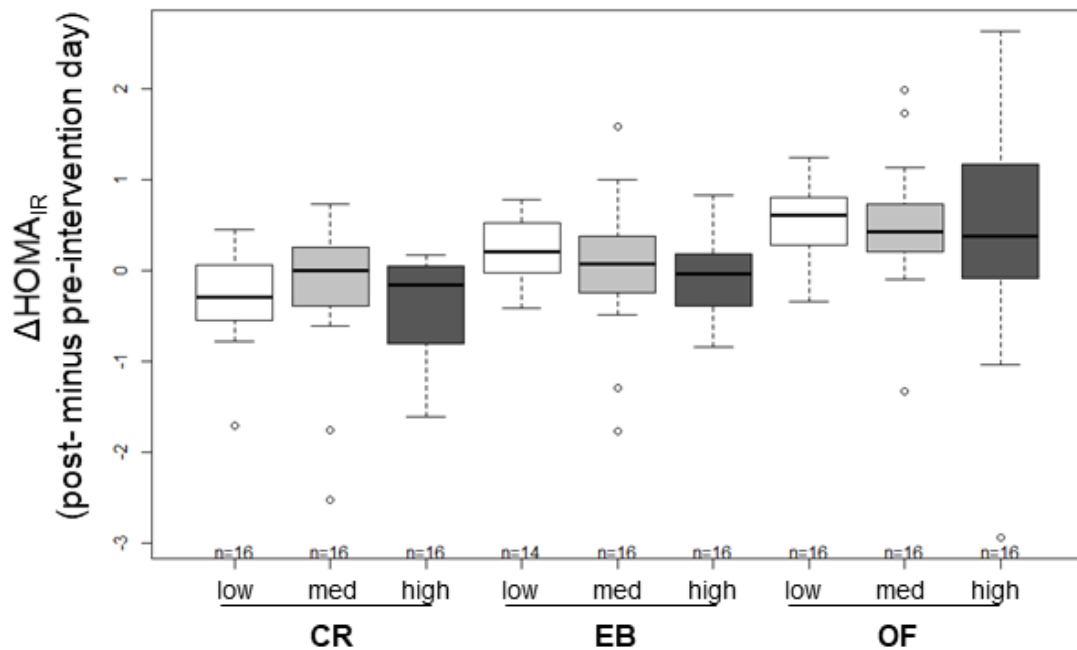
**TABLE 1:** Physical activity and daily energy-, macronutrient- and glycemic load intake during caloric restriction, zero energy balance and overfeeding with low, medium and high energy turnover.

	Caloric restriction			Energy balance			Overfeeding		
	low ET	med ET	high ET	low ET	med ET	high ET	low ET	med ET	high ET
<b>Physical activity</b>									
PAL	1.26 ± 0.06	1.52 ± 0.05	1.74 ± 0.07	1.30 ± 0.03	1.57 ± 0.04	1.75 ± 0.05	1.34 ± 0.05	1.55 ± 0.04	1.71 ± 0.05
Steps [counts/d]	545 ± 902	17,618 ± 557	34,527 ± 1,847	464 ± 912	17,710 ± 219	34,414 ± 1,539	436 ± 847	17,790 ± 382	34,821 ± 1,568
<b>Diet</b>									
Energy intake [kcal/d]	1,792 ± 144	2,156 ± 78	2,494 ± 90	2,390 ± 97	2,863 ± 72	3,325 ± 177	2,986 ± 99	3,570 ± 183	4,146 ± 320
CHO intake [g/d]	219.4 ± 20.8	255.1 ± 37.3	308.2 ± 12.0	292.7 ± 13.9	350.1 ± 6.0	406.5 ± 20.3	366.2 ± 11.8	437.2 ± 22.1	506.7 ± 37.9
Fat intake [g/d]	70.5 ± 4.9	84.7 ± 2.6	97.7 ± 5.0	93.7 ± 3.3	112.8 ± 3.9	131.0 ± 7.5	117.3 ± 3.9	140.8 ± 7.0	163.8 ± 12.5
Protein intake [g/d]	66.1 ± 4.0	79.4 ± 3.3	92.0 ± 5.6	88.4 ± 0.0	106.1 ± 5.6	120.3 ± 9.3	109.8 ± 3.0	131.0 ± 8.6	153.3 ± 13.0
Glycemic load [g/d]	124.0 ± 8.9	149.2 ± 4.7	173.8 ± 7.0	166.0 ± 6.5	197.8 ± 5.7	228.9 ± 14.1	206.4 ± 7.0	247.4 ± 13.0	285.9 ± 24.0

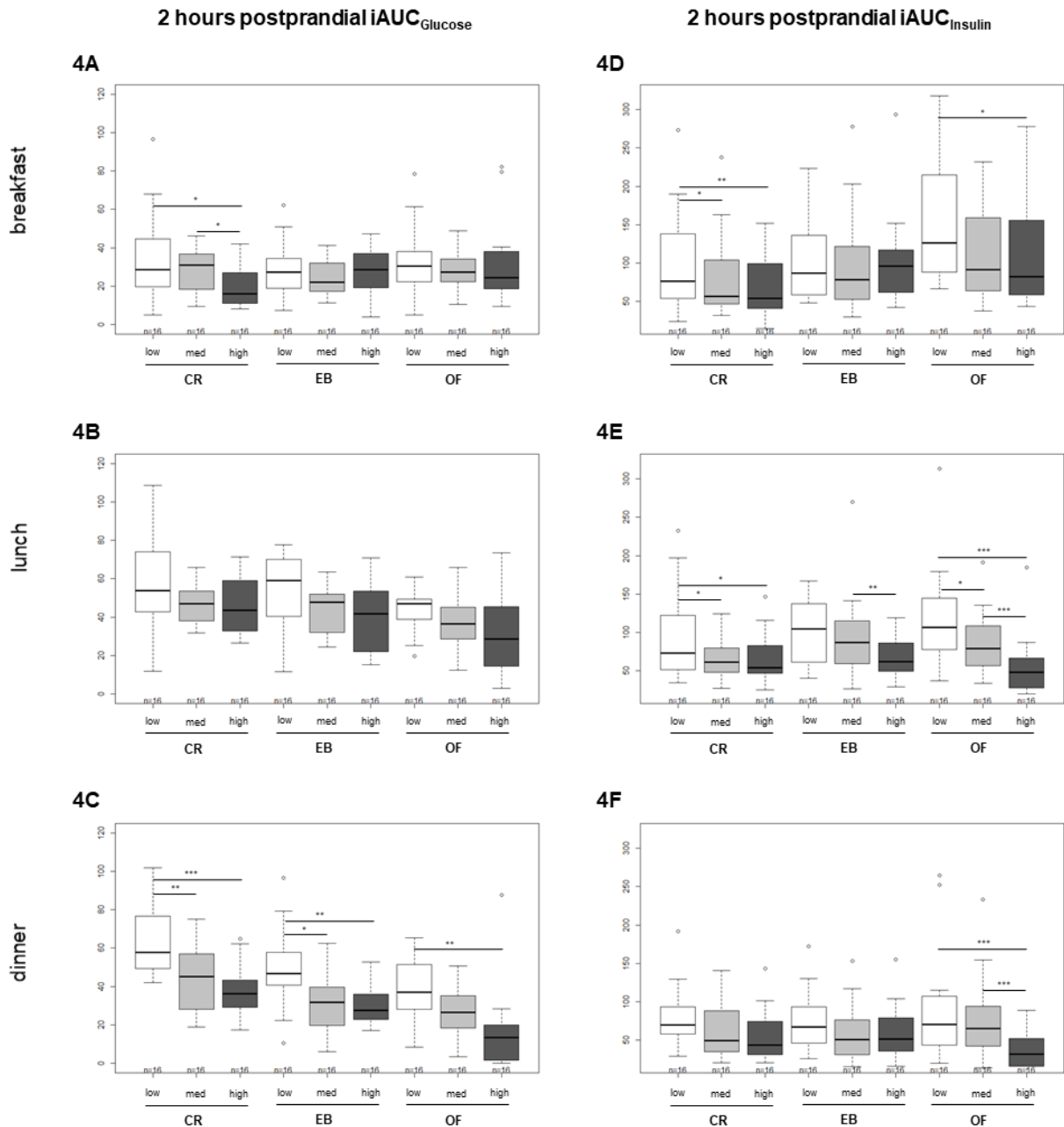
Values are means ±SDs; n=16; multiple contrast tests, results of physical activity were compared at the same level of ET between all energy balance conditions (all p>0.05) and within all energy balance conditions at three levels of ET (all significantly different at p<0.001); results of dietary intake were compared at the same level of ET between all energy balance conditions (all significantly different at p<0.001) and within all energy balance conditions at three levels of ET (all significantly different at p<0.001); ET, energy turnover; CHO, carbohydrate.



**FIGURE 2: Comparison between the different levels of ET (low, medium, high) at different energy balances (A) 18h iAUC<sub>cGM</sub> [mg / dl], n=16; (B) C-peptide excretions [µg / dl], n=15; values are means ±SDs; linear mixed model with multiple contrast tests, \* p<0.05 for comparison of ETs; CR, caloric restriction; EB, energy balance; OF, overfeeding; med, medium; ET, energy turnover.**



**FIGURE 3: Comparison of differences in HOMA-IR (post- minus pre-intervention day) between ETs (low, med, high) within different levels of energy balance (CR, EB, OF); values are means  $\pm$ SDs; linear mixed model with multiple contrast tests; fasting insulin sensitivity was improved with CR and impaired with OF (all  $p < 0.01$ );  $n = 14-16$ ; ET, energy turnover; med, medium; CR, caloric restriction; EB, energy balance; OF, overfeeding.**



**FIGURE 4: Comparison of 2 hours postprandial glucose (4A-C) and insulin (4D-F) iAUCs between the different levels of ET (low, medium, high) and energy balance (CR, EB, OF) separated by breakfast, lunch and dinner; values are means  $\pm$ SDs; linear mixed model with multiple contrast tests, \*  $p < 0.05$ , \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$  for comparison of ETs;  $n = 16$ ; ET, energy turnover; med, medium; CR, caloric restriction; EB, energy balance; OF, overfeeding.**

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## ***Chapter IV:***

### ***General discussion***

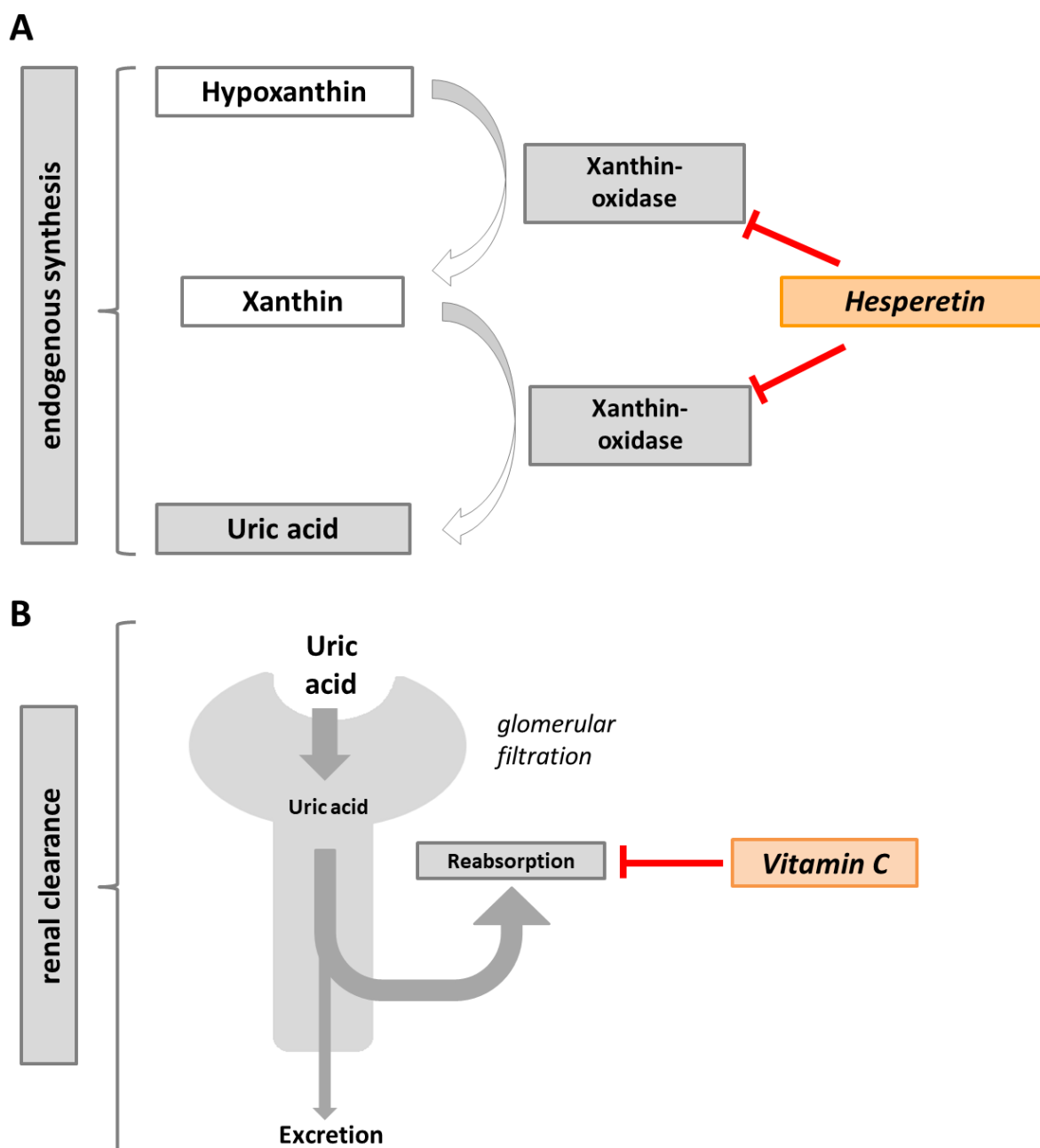
The present thesis investigated the impact of regular consumption of orange juice and cola on metabolic risk (uric acid levels and glycemia) in young healthy adults. Further, this thesis examined the impact of low intensity physical activity on postprandial and daylong glycemia in a controlled nutritional intervention study independent of energy balance.

*Impact of sugar-sweetened beverages on metabolic risk*

This thesis shows that regular consumption of cola for 14 days did not affect uric acid levels, whereas the consumption of orange juice even led to a decrease in uric acid. Contrary to our results, epidemiological studies found a positive association between the consumption of sugar sweetened beverages and an increased risk of gout (Choi & Curhan, 2008; Choi et al., 2010). Although fructose is known to induce purine degradation and thus increases uric acid levels (Fox & Kelley, 1972), uric acid levels remained unchanged after cola consumption. In the present study healthy young adults participated, suggesting that they were able to compensate for the increased ingested fructose load (fructose load per day: orange juice  $39.1 \pm 6.8$  g; cola  $47.4 \pm 8.2$  g). An acute fructose load of three different fructose-containing beverages (fructose load: 18 g, 17.5 g and 35 g for blueberry drink, coca cola and a fructose drink, respectively) did not increase uric acid levels among healthy individuals (Olofsson et al., 2019).

There are two main mechanisms that could explain the uric acid lowering effect by orange juice consumption (**Figure 2**). The first mechanism is a reduction of the endogenous synthesis of uric acid due to an inhibition of the key enzyme xanthine oxidase by hesperetin (Haidari et al., 2009). A previous *in vitro* study and a computer aided structural modeling showed that the chemical structure of flavonoids play a crucial role in their hypouricemic potential (Liang et al., 2002; Mo et al., 2007). More specifically, the planar structure with the hydroxyl groups on the carbon atoms can cause the competitive inhibition of xanthine oxidase by flavonoids interacting with the active site of xanthine oxidase. The inhibition of the enzyme xanthine oxidase thereby prevents the further reaction of hypoxanthine via xanthine to uric acid (Haidari et al., 2009). In orange juice, flavonoids mainly occur as flavanones in form of hesperidin and narirutin. Hesperetin is the deglycosylated form of hesperidin and represents the bioactive and -available catabolite (Aschoff et al., 2016).

The second proposed uric acid-lowering mechanism of orange juice is an enhanced clearance of uric acid, triggered by a competitive inhibition of renal reabsorption of uric acid by vitamin C at proximal tubules and/or an enhanced glomerular filtration (Huang et al., 2005). Since the excretion of uric acid was shown to be higher after the consumption of orange juice for two weeks (Chapter II, page 21), the second pathway partly explains the lower levels of uric acid. Orange juice consumed in the present study contained 186 mg / l



**Figure 2: Proposed mechanisms for lowering of uric acid by orange juice** (own figure)  
**(A) reduced endogenous synthesis of uric acid:** Inhibition of the key enzyme xanthine oxidase by hesperetin; according to Haidairi F et al.; *J Clin Biochem Nutr.* 2009 Nov; 45(3): 285–291.  
**(B) enhanced uric acid clearance:** Competitive inhibition of renal reabsorption of uric acid by vitamin C ( $447 \pm 78$  mg/d provided by orange juice) and/or enhanced glomerular filtration; according to Huang HY et al., *ARTHRITIS & RHEUMATISM*, Vol. 52, June 2005, 1843–1847.

hesperidin and 35 mg / 100 ml vitamin C (Chapter II, page 20), which results in a daily intake of  $237.6 \pm 41.5$  mg hesperidin and  $447.3 \pm 78.2$  mg vitamin C. It remains unclear to what extent and in which concentration the two ingredients hesperidin and vitamin C can improve

levels of uric acid or whether there is a synergistic effect of both substances. Odvina et al. performed a similar study aiming to investigate the comparative value of orange juice vs. lemonade in reducing stone-forming risk in thirteen volunteers with a mean age of 43 years (Odvina, 2006). After the consumption of 3 x 400 mL orange juice per day for a period of three weeks serum uric acid decreased compared to control ( $p < 0.0001$ ), whereas no change was observed after the consumption of lemonade. Contrary to our results, in the study of Odvina et al. no change in urinary excretion of uric acid was found in the three different phases. In this study, there was no information on the content of vitamin C or secondary plant metabolites in the orange juice.

The second important result of this study was that daylong glycemia and glucose variability were higher when consuming cola compared to orange juice. Unlike cola, orange juice contains small amounts of fat, protein, fiber, secondary plant metabolites and vitamin C, of which some may positively affect glucose metabolism (see **Figure 3**). The amount of fiber in orange juice with pulp is less than 1 g per 100 ml and thus very low. By changing the GI of orange juice, fiber and/or other ingredients, like small amounts of fat or protein, could have mitigated the blood glucose increasing effect of the sugar load of the juice itself. In addition, flavonoids might impact the glycemic response to orange juice (for review see (Hanhineva et al., 2010)). There are many possibilities how flavonoids could influence the glucose metabolism of healthy people: An *in vivo* study in rats with *in vitro* experiments in intestinal tissue showed that the activity of disaccharidases can be inhibited by flavonoids leading to impaired glucose absorption (Fontana Pereira et al., 2011). The flavonoid rutin was shown to inhibit maltase activity in healthy rats and in diabetic rats and therefore was able to reduce postprandial glycemia in these animals. Naringenin, a flavonoid present in citrus fruits and juices, has been shown to significantly inhibit intestinal glucose uptake in rats and rabbits by using purified intestinal brush border membrane vesicles and everted intestinal sleeves (Li et al., 2006). Glucose uptake was reduced by more than 60% in both the intestinal and renal brush border membrane vesicles of diabetic rats. In addition, some hesperidin derivatives have been evaluated in *in vitro* and *in vivo* studies regarding the  $\alpha$ -glucosidase inhibition (Zhang et al., 2012). Several compounds of hesperidin have been shown to cause anti-hyperglycemic effects.

In addition, a study in which rats were fed with a flavonoid-rich diet with extract of *Chromolaena odorata* – a plant with anti-diabetic properties according to folklore (Adedapo et al., 2016) – revealed a 130.7 % increase in circulating GLP-1 versus control (Omotuyi et al., 2018). Two phytochemicals were identified as putative anti-diabetic because they led to an enhanced expression of GLP-1.

Furthermore, a recent controlled clinical study with 10 healthy young women showed a positively modulated composition and metabolic activity of gut microbiota (i.e. increase in fecal *Bifidobacterium* spp. and *Lactobacillus* spp) after two months of regular orange juice consumption. This consumption might have contributed to the improvement in glucose levels and insulin sensitivity that was observed in the study (Lima et al., 2019). However, in the present study neither orange juice nor cola consumption was associated with significant changes in fecal microbiota diversity (Shannon diversity index) or taxonomic composition (Bray-Curtis dissimilarity). Since the intervention period in our study was only two weeks, this might have been too short to impact gut microbiota.

As protection against prediabetes and the development of diabetic complications, two studies in a diabetic mouse model have shown positive effects of flavonoids (Jung et al., 2004, 2006). In these studies, hesperidin has been shown to prevent hyperglycemia by increasing hepatic glucokinase activity and glycogen concentration and/or by lowering hepatic gluconeogenesis. Likewise, a randomized, double-blinded study showed a reduction in fasting blood glucose levels in patients with type 2 diabetes after an eight-week supplementation of 500 mg hesperidin per day (Eghtesadi et al., 2016). In the present study, the intake of hesperidin via orange juice was  $237.6 \pm 41.5$  mg per day, which is about half of the dose compared to the study of Eghtesadi et al. and could thus have possibly led to the slightly lower glycemia and glucose variability with consumption of orange juice compared to cola.



**Figure 3: Ingredients of orange juice (hohes C, milde Orange mit Fruchtfleisch) and Coca Cola without caffeine (French: sans caféine); French: glucides, carbohydrates; dont sucres, of which sugar (pictures by Franziska Büsing, 2016).**

Besides the potential health-supporting ingredients, orange juice however contains almost as much sugar as cola (orange juice: 8.8 g vs. cola: 11.1 g). Therefore, another explanation for the findings of this thesis could be that glycemia was not lowered after the consumption of orange juice, but was increased after the consumption of cola. Consistent with a higher 24h-glycemia during the consumption of cola, a reduced 24h-insulin secretion was found with cola intervention compared to orange juice. An impaired insulin secretion may explain the higher 24h-glycemia after cola- compared to orange juice consumption. A study in rodents has shown that chronic consumption of cola led to a reduction in  $\beta$ -cell size (Otero-Losada et al., 2015). However,  $\beta$ -cell damage is unlikely to explain the 45 % lower C-peptide excretion with cola- compared to orange juice consumption in the present study. If cola consumption had such a toxic effect on  $\beta$ -cells, regular intake of high amounts of cola would drastically increase the risk of type 2 diabetes. However, an epidemiological study found an increased incidence of gestational diabetes in women with regular cola consumption, but

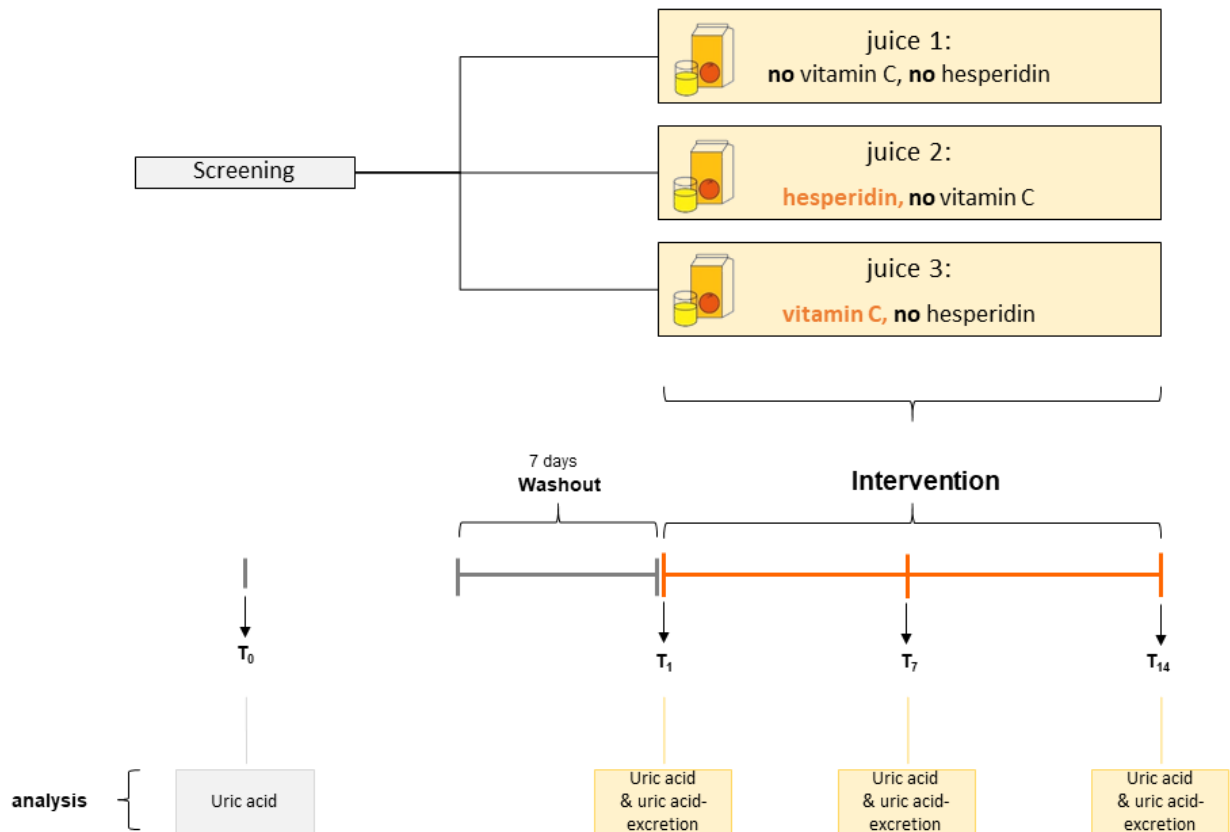


not after consuming other sodas and suggested a link to the caramel coloring contained in cola drinks which needs to be further investigated (Chen et al., 2009).

Since consumption of SSBs is known to increase the risk of weight gain (Hu & Malik, 2010), type 2 diabetes (O'Connor et al., 2015) and dental caries (Bernabé et al., 2014), the consumption should be restricted. Because of a favorable composition (secondary plant products and vitamin C), the German Nutrition Society (DGE) allows to replace a portion of fruit per day with one glass of fruit juice (DGE, 2007). Fruit juice can therefore help to reach the recommended five servings of fruits and vegetables a day. Situational prevention in the form of state regulations like the introduction of a taxes on sugary drinks or a ban of advertising for specific unhealthy drinks should be considered in order to reduce the consumption of SSBs especially in young people who consume the highest amounts of SSBs (Max Rubner-Institut, 2008). The introduction of a sugar tax has already been shown to be successful in other countries (Bascuñán & Cuadrado, 2017; Silver et al., 2017).

*OUTLOOK.* Since hyperuricemia is usually not treated by medication, the lowering of serum uric acid by the consumption of orange juice could be a promising preventive method.

As the present study only examined the uric acid-lowering effect of orange juice on healthy and young volunteers, a study in patients with already elevated uric acid levels needs to be implemented in order to confirm similar effects on patients at risk. However, when consumed in large doses, orange juice, like other sugar-containing beverages, is a potential risk factor for the development of obesity (Hägele et al., 2018). In order to ensure that the intake of one glass of orange juice (250 ml) per day is not exceeded, the juices in this study could be enriched with hesperidin and vitamin C to reach comparable concentrations like in the previous study. In a planned follow-up study, the effects of hesperidin and vitamin C from orange juice on uric acid levels will be investigated in subjects with elevated uric acid levels ( $> 6.8$  mg / dl) using three different fruit juice beverage variants (**Figure 4**). In a 14-day intervention phase (T1-T14), participants are asked to consume 250 ml of orange juice (either juice 1 / 2 / 3) once a day while maintaining their normal dietary habits. After 7 days (T7) and at the end of the study (T14), uric acid levels and uric acid excretion of the subjects will be examined.



**Figure 4: Schematic study protocol of a planned follow-up study**

*Impact of physical activity on glycemia independent from changes in energy balance*

The energy turnover study has shown that low intensity physical activity performed after meals led to lower postprandial levels of glucose and insulin independent of energy balance but had no impact on 24-hour glucose metabolism with zero energy balance, caloric restriction and overfeeding. Since the study was performed in healthy subjects with normal basal glucose levels, no effect on fasting glycemia subjects was expected.

The observed effects of physical activity on postprandial glycemia mainly were due to acute modulation of non-insulin-dependent glucose uptake into the muscle. Any increase in physical muscle fiber tension increases the transmembrane glucose transport by stimulating the translocation of glucose transporters GLUT4 from intracellular compartments such as the endoplasmic reticulum to the muscle cell membrane (Stanford & Goodyear, 2014). This causes the blood glucose lowering effect during physical activity. This process is mediated independently of the regular glucose uptake pathway via the insulin receptor and could be used to lower postprandial glycemia.

Previous studies have already shown that exercise, i.e. a planned physical activity mostly with medium to high intensity, improved glucose metabolism by increasing glucose uptake and improving insulin sensitivity (for review see (Bird & Hawley, 2017)). A meta-analysis found that even low intensity physical activity like walking was associated with a 15 % decrease in the relative risk of type 2 diabetes (Aune et al., 2015). Other studies have shown that low intensity walking was enough physical effort to improve postprandial glycemia (Manohar et al., 2012; Nygaard et al., 2009). The results of the energy turnover study confirm these findings and show that walking with a speed of 4 km/h on the treadmill for 3 x 55 min or 3 x 110 min per day is sufficient to achieve a beneficial effect on postprandial glycemia. This suggests that low intensity physical activity is a promising strategy to improve regulation of glucose metabolism.

In addition to intensity of physical activity, timing plays a major role in regulation of glycemia. Postmeal physical activity demonstrated to be a more effective method to manage postprandial high levels of blood glucose when compared with premeal physical activity (Colberg et al., 2009). For optimal efficacy physical activity should start about 30 minutes after a meal (Chacko, 2014). In line with these findings, all physical activity in the present study was performed in a post-meal situation, starting 40 minutes after beginning of the meal.

Postprandial insulin sensitivity was improved by low intensity physical activity, especially after lunch and dinner. Since insulin sensitivity is subject to a circadian rhythm, and is lower in the evening (Saad et al., 2012), the greatest potential for improving insulin sensitivity by physical activity is in the evening. Current exercise guidelines for the prevention and treatment of diabetes, which have been developed by the Deutsche Diabetes Gesellschaft (DDG), do not mention the relevance of exercise timing in relation to meal ingestion (Bundesärztekammer et al., 2013). Following the guidelines of the American College of Sports Medicine (ACSM), the American Diabetes Association (ADA) and the Scottish Intercollegiate Guidelines Network (SIGN), a specified 3-step-program is also recommended by the DDG. This concept includes advice for increasing daily unstructured physical activity (step 1), structured exercise programs including aerobic endurance training, and/or strength training (step 2) and complementary concepts aiming to maintain coordination, dexterity, flexibility and responsiveness (step 3) for patients with type 2 diabetes (Bundesärztekammer et al., 2013; Colberg et al., 2010; Colberg et al., 2010; Inzucchi et al., 2012). The aerobic endurance training is recommended to be performed at least three days per week with a total of 150 minutes and at least moderate intensity. A controlled intervention study among patients with diabetes showed that a combination of aerobic and resistance training improved HbA(1c) levels (Church et al., 2010).

Several studies have shown that a positive energy balance is associated with impaired insulin sensitivity (Brøns et al., 2009; Cornier et al., 2006; Wang et al., 2001), and a negative energy balance leads to an improvement of postprandial glycemia in patients with non-insulin-dependent diabetes (Larsen et al., 1997). One study showed an impaired insulin sensitivity after 3 days of overfeeding (150% habitual intake) combined with a reduction in the steps in healthy young man ( $\leq 1500$  steps per day (Knudsen et al., 2012)). Participants gained weight after 7 days of the experiments and fat mass after 14 days, whereas impairments in insulin sensitivity already occurred after three days before measureable changes in body composition. In another study under conditions of overfeeding a single bout of exercise decreased the postprandial insulin response compared to overfeeding alone (Hagobian & Braun, 2006). In addition, Walhin et al. also showed that vigorous-intensity exercise counteracts most of the effects of a positive energy balance, created by short-term overfeeding and reduction in physical activity (Walhin et al., 2013). However, so far no studies have examined the influence of physical activity on glucose metabolism

separate from the influence of energy balance. The concept of energy turnover allows to investigate the impact of different levels of physical activity at the same energy balance. The improvement of postprandial glycemia at higher levels of energy turnover was found with caloric restriction, zero energy balance, and overfeeding, and thus shows that this effect is independent of energy balance. These results also suggest that physical activity may ameliorate the negative effects of a positive energy balance. Since our study only performed a short-term intervention over 24 hours, the transfer of these results to chronic effects is limited.

*OUTLOOK.* The World Health Organization developed recommendations that addresses the link between the frequency, duration, intensity, and total amount of physical activity, which is needed to prevent prediabetes and diabetes (WHO, 2010). Physical activity was defined as recreational or leisure-time physical activity, transportation (walking or cycling), household activities, gaming, sports, or planned exercise. According to this WHO-recommendation, adults aged 18-64 years should perform at least 150 minutes of moderate-intensity aerobic physical activity per week or a combination of moderate- and vigorous-intensity activity. A bout of aerobic activity should be at least of 10 minutes duration.

These recommendations can also be applied to older adults with disabilities, or overweight people. Specific health risks, individual training levels, or other limitations, therefore, need to be considered and have to be adjusted for each individual.

150 minutes of moderate-intensity aerobic physical activity per week seem to be a very small amount of physical activity, in order to achieve preventive outcomes regarding diabetes. In the present study an improvement in postprandial glycemia was partially achieved with a PAL of 1.5, but the effects were more pronounced with a PAL of 1.8. In order to achieve a PAL of 1.8, the subjects had to walk on the treadmill 3 x 110 min at 4 km/h. The fact that the subjects had been in a 9 m<sup>2</sup> metabolic chamber throughout the whole study, limited their physical activity to only treadmill activity. In order to achieve a high level of energy turnover in this study, participants had to do  $\geq 34,500$  steps per day. This amount of steps would be significantly less in their everyday life with habitual household activities, and without the spatial restriction.

The 2005 Dietary Guidelines for Americans divided recommendations to separate advice about prevention of chronic diseases like diabetes from advice about the physical activity required for the prevention of weight gain (US Department of Health and Human Services, 2008). According to these guidelines, 30 minutes of moderate-intensity physical activity per day could already provide protection against the development of chronic diseases, whereas there is less well documented reference for prevention of unhealthy weight gain. However, the impact of physical activity on glucose metabolism should be divided into acute and long-term effects. To achieve an immediate improvement in postprandial glycemia of middle-aged women, a 15 minutes postmeal walk with low intensity was sufficient (Nygaard et al., 2009). In addition, Larsen et al. revealed that a postprandial glycemia-lowering effect achieved through moderate intensity physical activity does not persist during and after the following meal (Larsen et al., 1997). Since the postprandial effect did not last long and could no longer be observed after the next meal, it is an acute effect. On the other hand, long-term interventions improve physical fitness and mitochondrial content, and thus improves glucose metabolism in the long-term (Meex et al., 2010).

In conclusion, health effects of commonly consumed sugar-containing beverages like orange juice or cola are not fully explained by their sugar content. While regular consumption of cola had adverse effects on glycemia, consumption of orange juice may protect against hyperuricemia by containing the potential uric-acid lowering ingredients vitamin C and hesperidin (Chapter II).

Postprandial glucose and insulin levels in healthy adults are improved by performing low intensity physical activity after meals independently of changes in energy balance. Since higher postprandial glycemia, even below the diabetic threshold, has been shown to be a risk factor for cardiovascular disease, walking after meals could be an advisable preventive strategy to prevent or postpone the onset of diabetes (Chapter III).

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## ***Chapter V:***

### ***Limitations of methods***

The studies, discussed in this thesis, analyze insulin resistance by HOMA-index, glycemia by continuous interstitial glucose monitoring, and insulin secretion by C-peptide excretion during controlled nutritional interventions. Although these methods were chosen carefully, there are some limitations that need to be addressed.

*ASSESSMENT OF DAYLONG GLYCEMIA.* To monitor the subjects' glucose levels continuously during multi-day intervention studies, participants wore a continuous glucose monitoring (CGM) device. CGM is a method based on glucose concentrations measured every 5 minutes in the interstitial fluid by a tiny subcutaneous glucose sensor (DE Dexcom 2019). The glucose sensor is an amperometric enzyme sensor. It works as an electrochemical method for the quantitative determination of chemical substances and selectively quantifies glucose at the sensor platinum thread in the tissue (Oliver et al. 2009). The underlying mechanism is an enzymatic conversion of glucose together with oxygen by glucose oxidase via gluconolactone to gluconic acid and hydrogen peroxide (Wang and Lee 2015). The resulting hydrogen peroxide is oxidized electrochemically at a potential of 600 mV at a platinum electrode whereby a current flow is possible. This flow of electricity is registered by a highly sensitive ampere meter, whereby the measured flow is proportional to the concentration of glucose.

Since glucose needs time after a meal in order to be taken up into the blood, and to be transported to the interstitium, there is a physiological time lag between the two compartments (Cobelli et al. 2016). Interstitial fluid (ISF) glucose thereby is not just blood glucose shifted-in-time, but exhibits a complex pattern that depends on the two metrics “glucose lag” and “time lag”. The “glucose lag” is characterized by the time course of glucose differences between ISF and blood glucose at each time point and “time lag” (the time course of time differences when ISF glucose is equal to blood glucose). Based on these findings, the interstitial glucose should be evaluated with caution. However, the deviation of interstitial glucose levels from blood glucose can be minimized by regularly calibrating the CGM against fasting capillary blood, which was done at least twice a day in the present studies. Also, the study protocol of the energy turnover study in this thesis is based on an intraindividual comparison of the subjects with themselves using the same sensor during different energy turnover days. The autonomous use, easy handling, and the ability to provide a continuous, subject-independent monitoring of blood glucose levels, makes the CGM device ideal for the use in clinical trials and research settings (Liao and Schembre 2018).

*ASSESSMENT OF 24-H INSULIN SECRETION.* Daylong insulin secretion was assessed by 24-h urinary C-peptide excretion via luminescence immunodetection. After cleavage of

proinsulin in the pancreatic  $\beta$ -cells, C-peptide is excreted into the urine and can be detected in equimolar ratio with insulin (Wahren et al. 2012). Analyzing urinary C-peptide excretion in order to determine 24-h insulin secretion is well established under steady-state conditions. The kidneys filter about one quarter of the arterial C-peptide (Zavaroni et al. 1987). Afterwards, most of it (~85%) is taken up again in the renal tubulus and then degraded to amino acids, while 15% is excreted with the urine. Contrary, at non-steady-state conditions achieved through caloric restriction or overfeeding, this method could be compromised (Polonsky et al. 1986). In addition, hepatic extraction and metabolism of insulin, and renal clearance of C-peptide can be affected by hepatic diseases or renal insufficiency. Both of these influences could lead to incorrect calculations of insulin secretion. Although our studies were not performed under steady state conditions, we can rule out impaired kidney function and other metabolic impairments by choosing healthy volunteers.

*CALCULATION OF BASAL INSULIN SENSITIVITY VIA HOMA<sub>IR</sub>*. Homeostatic Model Assessment-insulin resistance ( $HOMA-IR = \text{fasting glucose (mg/dl)} \times \text{fasting insulin } (\mu\text{U/ml}) / 405$ , Matthews et al. 1985) was used to calculate fasting insulin sensitivity (Matthews et al. 1985). HOMA-IR is a method for primarily measuring hepatic insulin sensitivity rather than peripheral insulin sensitivity (Hoffman 2008). In contrast to glucose clamp technique, it should be recognized that HOMA is validated under fasting steady state conditions and is not intended to give information about the stimulated state (Wallace et al. 2004). During the energy turnover study these assumptions were not met. For example, during caloric restriction fasting levels of insulin can decrease without necessarily affecting insulin sensitivity. The non-steady state conditions achieved through the controlled overfeeding and caloric restriction may therefore have biased the results.

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## **Summary**

In epidemiological studies consumption of sugar-containing beverages is associated with an increased risk of overweight, type 2 diabetes, and hyperuricemia. The aim of the study was to investigate whether orange juice has the same negative effects on metabolic risk as cola. A two-week crossover intervention study compared the effect of consumption of 20% of individual energy requirement as caffeine-free cola and orange juice on metabolic risk among 26 healthy young adults. Despite the same sugar intake (orange juice  $112 \pm 20$  g/day vs. cola  $114 \pm 20$  g/day), the consumption of cola resulted in a higher daylong glycemia as well as a higher glucose variability and a lower C-peptide excretion as marker of insulin secretion. Consumption of cola did not affect uric acid levels, whereas orange juice led to a decrease in serum uric acid levels and increased uric acid excretion. The improved uric acid excretion could be explained by the high vitamin C content of orange juice. Furthermore, hesperidin, the main flavonoid of orange juice, could have led to lower uric acid levels by decreasing endogenous synthesis of uric acid. Regular consumption of orange juice could therefore have protective and therapeutic effects on hyperuricemia and gout. The results of this study show that epidemiological associations need to be verified by intervention studies, and that the evaluation of health-related effects of sugar-containing beverages cannot be confined to their sugar content.

In a second study, the impact of low intensity physical activity (walking at 4 km/h) on postprandial glycemia was studied among 16 healthy young adults independent of energy balance. The study design was created based on the energy turnover model. Energy turnover is defined as the level of energy balance. A low energy turnover is characterized by a low level of physical activity (low energy requirement) with a correspondingly low energy intake. Subjects were examined under three different daylong activity levels with (a) no treadmill activity, (b) 3 x 55 min, or (c) 3 x 110 min walking on a treadmill. This protocol was conducted under conditions of caloric restriction (-25% of total energy requirement), zero energy balance (energy consumption = energy intake), as well as overfeeding (+25% of total energy requirement). Postprandial glucose and insulin levels were improved by increasing levels of postmeal physical activity. This reduction in postprandial glycemia was true for zero energy balance, caloric restriction, and overfeeding, and was thus independent of energy balance and presumably due to non-insulin-mediated glucose uptake. On the

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other hand, low intensity physical activity had no effect on 24-h glycemia in healthy normoglycemic subjects. The results of this study show that a moderate increase in daily activity is sufficient to improve postprandial glycemia.



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## **Zusammenfassung**

Der Verzehr zuckerhaltiger Getränke wird in epidemiologischen Studien mit einem erhöhten Risiko für Übergewicht, Typ 2 Diabetes und Hyperurikämie assoziiert. Ziel der ersten Studie dieser Arbeit war es herauszufinden, ob Orangensaft dieselben negativen Effekte auf das metabolische Risiko aufweist, wie Cola. In einer zweiwöchigen *Cross-over* Interventionsstudie wurde der Einfluss des regelmäßigen Verzehrs von Orangensaft und Cola auf das metabolische Risiko von 26 gesunden jungen Erwachsenen untersucht. Die Probanden tranken hierzu für zwei Wochen 20% ihres individuellen Energiebedarfs in Form von koffeinfreier Cola oder Orangensaft. Der Verzehr von Cola führte im Vergleich zu Orangensaft, trotz gleicher Zuckeraufnahmemenge (Orangensaft  $112 \pm 20$  g/Tag vs. Cola  $114 \pm 20$  g/Tag), zu einer höheren Tagesglykämie sowie zu einer höheren Glucosevariabilität und einer geringeren C-Peptidausscheidung als Marker der Insulinsekretion. Der Konsum von Orangensaft führte außerdem zu einer Reduktion der Harnsäurespiegel und einer erhöhten Harnsäureausscheidung. Die verbesserte Harnsäureausscheidung könnte durch den hohen Vitamin C-Gehalt des Orangensafts erklärt werden. Darüber hinaus ist eine verminderte Harnsäuresynthese durch Hesperidin, das Hauptflavonoid des Orangensafts, als Ursache für eine Senkung des Harnsäurespiegels möglich. Der Verzehr von Cola beeinflusste die Harnsäurespiegel nicht. Ein regelmäßiger Konsum von Orangensaft könnte daher protektive und therapeutische Effekte im Hinblick auf Hyperurikämie und Gicht haben. Die Ergebnisse dieser Studie zeigen, dass epidemiologische Zusammenhänge durch Interventionsstudien überprüft werden müssen und, dass der Effekt zuckerhaltiger Getränke auf die Gesundheit nicht ausschließlich auf den Zuckergehalt beschränkt werden kann.

In einer zweiten Studie wurde der Einfluss von körperlicher Aktivität bei niedriger Intensität (Gehen bei 4 km/h) auf die postprandiale Glykämie unabhängig von der Energiebilanz bei 16 gesunden jungen Erwachsenen untersucht. Hierzu diente das Energie-Umsatz-Modell. Der Energie-Umsatz ist definiert als Niveau der Energiebilanz, d.h. ein niedriger Energieumsatz entspricht demnach einem geringen Maß an körperlicher Aktivität (niedriger Energiebedarf) mit einer korrespondierend niedrigen Kalorienaufnahme. Die Probanden wurden hierfür unter drei verschiedenen Aktivitätsniveaus untersucht: (a) keine Laufbandaktivität, (b) 3 x 55 min oder (c) 3 x 110 min gehen auf dem Laufband. Dieses

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Protokoll wurde sowohl bei Kalorienrestriktion (-25% des Gesamtenergiebedarfs), ausgeglichener Energiebilanz (Energieverbrauch = Energieaufnahme), als auch bei Überernährung (+25% des Gesamtenergiebedarfs) durchgeführt. Es konnte gezeigt werden, dass durch die Steigerung der körperlichen Aktivität nach den Mahlzeiten, Verbesserungen der postprandialen Glucose- und Insulinspiegel erzielt werden konnten, was vermutlich durch eine Verbesserung der Insulin-unabhängigen Glukoseaufnahme erklärt wird. Da die Senkung der postprandialen Glykämie sowohl bei ausgeglichener Energiebilanz, als auch bei Kalorienrestriktion und Überernährung nachweisbar war, ist sie unabhängig von der Energiebilanz. Die körperliche Aktivität bei niedriger Intensität hatte hingegen keinen Effekt auf die 24h Glykämie bei gesunden normoglykämien Probanden. Die Ergebnisse dieser Studie zeigen, dass für eine Verbesserung der postprandialen Glykämie bereits eine moderate Steigerung der Alltagsaktivität ausreicht.

## ***Appendix***

Einwilligungserklärung Orangensaft Studie

Ethikvotum Orangensaft Studie

Declaration of Co-Authorship Orangensaft Studie

Einwilligungserklärung Energy-Flux Studie

Ethikvotum Energy-Flux Studie

Declaration of Co-Authorship Energy-Flux Studie

Curriculum vitae

Danksagung

## Schriftliche Einwilligungserklärung

Hiermit erkläre ich mich bereit, an der oben genannten Studie teilzunehmen. Ich bin über Zweck, Ziele und Durchführung der Studie informiert und aufgeklärt worden und habe sie verstanden. Darüber hinaus hatte ich Gelegenheit, Fragen zum Verständnis und zur Durchführung zu stellen, welche zufriedenstellend beantwortet wurden. Diese Einwilligung ist freiwillig und kann von mir jederzeit ohne die Angabe von Gründen formlos widerrufen werden ohne, dass mir daraus Nachteile entstehen. Eine Kopie des Informationsblattes habe ich erhalten.

Mir wurde versichert, dass die Vorschriften über die ärztliche Schweigepflicht und den Datenschutz im Rahmen dieser Studie eingehalten werden und dass nur pseudonymisierte (=verschlüsselte) Datenbögen ohne Namensnennung weitergegeben werden. Mir wurde erklärt, dass mein Name dabei durch einen Zifferncode, ohne Angabe von Initialen oder Geburtsdaten, ersetzt wird und lediglich der Studienkoordinator und der Studienleiter Zugriff auf die Referenzliste haben, die die erhobenen Daten einer Person zuordnet.

**Ich bin mit der beschriebenen Datenerhebung, -verarbeitung und –weitergabe einverstanden. Mir wurde versichert, das Dritte keinen Einblick in Originalunterlagen erhalten**

\_\_\_\_\_  
Name des Teilnehmers (Druckbuchstaben)

\_\_\_\_\_  
Unterschrift des Teilnehmers

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Datum, Ort

## Studienkoordinator/Studienpersonal

Ich habe dem/der oben genannten Teilnehmer/-in die durchzuführende Studie im Detail erklärt und alle aufkommenden Fragen zu seiner/ihrer Zufriedenheit erklärt. Eine Kopie der Probandeninformation sowie der Einwilligungserklärung wurden dem/der Teilnehmer/-in ausgehändigt.

Prof. Dr. Anja Bosy-Westphal

\_\_\_\_\_  
Name des aufklärenden Arztes

\_\_\_\_\_  
Unterschrift der aufklärenden Person des Studienpersonals

\_\_\_\_\_  
Datum, Ort

**LANDESÄRZTEKAMMER BADEN-WÜRTTEMBERG**

KÖRPERSCHAFT DES ÖFFENTLICHEN RECHTS

**ETHIK-KOMMISSION**



Landesärztekammer Baden-Württemberg • Postfach 700361 • 70573 Stuttgart

20.04.2016

Universität Hohenheim  
Institut für Ernährungsmedizin 180  
Frau Franziska Büsing  
Fruwirthstr. 12  
70593 Stuttgart

Ansprechpartnerin:  
irmgard.ossmann@laek-bw.de  
Tel.: 0711 76989-97  
Fax: 0711 76989-856

**Internes Aktenzeichen:** F-2016-015  
**Titel:** Einfluss von regelmäßigem Orangensaftkonsum auf die Blutzuckerregulation und den antioxidativen Status bei gesunden Probanden

**Antrag vom:** 28.02.2016

Sehr geehrte Frau Büsing,

besten Dank für Ihr Schreiben vom 18.04.2016 samt Anlagen in Antwort auf unser Schreiben vom 30.03.2016.

Dem Hinweis der Ethik-Kommission ist Rechnung getragen.

Gegen die weiteren Änderungen in dem aktualisierten Prüfplan und aktualisierten Probandeninformation bestehen keine Bedenken.

Mit freundlichen Grüßen

Dr. med. G. Hook  
Stellvertretender Vorsitzender der Ethik-Kommission

**Eingereichte Unterlagen:**

**Mit Schreiben vom 18.04.2016**

- Prüfplan Version 1.2 vom 19.04.2016
- Probandenaufklärung Version 1.2 vom 19.04.2016

<b>C</b>	<b>A</b>	<b>U</b>	Christian-Albrechts-Universität zu Kiel	Agrar- und Ernährungs- wissenschaftliche Fakultät
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### Declaration of co-authorship

If a dissertation is based on already published or submitted co-authored articles, a declaration from each of the authors regarding the part of the work done by the doctoral candidate must be enclosed when submitting the dissertation.

#### 1. Doctoral candidate

**Name:** Franziska Büsing






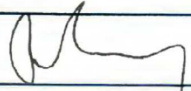
#### 2. This co-author declaration applies to the following article:

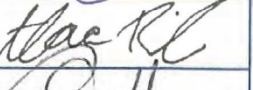
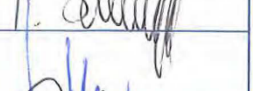
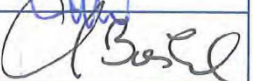
**High intake of orange juice and cola differently affects metabolic risk in healthy subjects**

The extent of the doctoral candidate's contribution to the article is assessed on the following scale:

- A. Has contributed to the work (0-33%)
- B. Has made a substantial contribution (34-66%)
- C. Did the majority of the work independently (67-100%)

3. Declaration on the individual phases of the scientific work (A,B,C)	Extent
<b>Concept:</b> Formulation of the basic scientific problem based on theoretical questions which require clarification, including a summary of the general questions which, it is assumed, will be answerable via analyses or concrete experiments/investigations	A
<b>Planning:</b> Planning of experiments/analyses and formulation of investigative methodology, including choice of method and independent methodological development, in such a way that the scientific questions asked can be expected to be answered	B
<b>Execution:</b> Involvement in the analysis or the concrete experiments/investigation	C
<b>Manuscript preparation: Presentation, interpretation and discussion of the results obtained in article form</b>	C

4. Signature of all co-authors		
Date	Name	Signature
25.03.2019	Franziska A. Hägele	
29.03.2019	Alessa Nas	
12.04.2019	W. Florian Fricke	
29.03.2019	Tobias Pöhl	
12.04.19	Elisabeth Dörner	
12.04.19	Daniel Podlesny	

15.04.2019	Laura-Verena Döbert	
12.04.2019	Alena Fricker	
30.3.2019	Julian Aschoff	
30.3.2019	Ralf Schweiggert	
30.3.2019	Reinhold Carle	
25.3.2019	Anja Bosy-Westphal	

5. Signature of doctoral candidate		
Date	Name	Signature
25.03.19	Franziska Büsing	F. Büsing

## Schriftliche Einwilligungserklärung

Hiermit erkläre ich mich bereit, an der oben genannten Studie teilzunehmen. Ich bin über Zweck, Ziele und Durchführung der Studie informiert und aufgeklärt worden und habe sie verstanden. Darüber hinaus hatte ich Gelegenheit, Fragen zum Verständnis und zur Durchführung zu stellen, welche zufriedenstellend beantwortet wurden. Diese Einwilligung ist freiwillig und kann von mir jederzeit ohne die Angabe von Gründen formlos widerrufen werden ohne, dass mir daraus Nachteile entstehen. Eine Kopie des Informationsblattes habe ich erhalten.

**Mir wurde versichert, dass die Vorschriften über die ärztliche Schweigepflicht und den Datenschutz im Rahmen dieser Studie eingehalten werden und dass nur pseudonymisierte (=verschlüsselte) Datenbögen und Blutproben ohne Namensnennung weitergegeben werden. Mir wurde erklärt, dass mein Name dabei durch einen Nummern- und Buchstabencode, ohne Angabe von Initialen oder Geburtsdaten, ersetzt wird und lediglich der Studienkoordinator und der Studienleiter Zugriff auf die Referenzliste haben, die die erhobenen Daten einer Person zuordnet.**

**Ich bin mit der beschriebenen Datenerhebung, -verarbeitung und -weitergabe einverstanden. Mir wurde versichert, das Dritte keinen Einblick in Originalunterlagen erhalten.**

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Unterschrift des Teilnehmers

\_\_\_\_\_  
Datum, Ort

## Studienkoordinator/Studienpersonal

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Prof. Dr. oec. troph. Dr. med. Anja Bosy-  
Westphal

\_\_\_\_\_  
Name des aufklärenden Arztes

\_\_\_\_\_  
Unterschrift der aufklärenden Person des  
Studienpersonals

\_\_\_\_\_  
Datum, Ort



## LANDESÄRZTEKAMMER BADEN-WÜRTTEMBERG

KÖRPERSCHAFT DES ÖFFENTLICHEN RECHTS

### ETHIK-KOMMISSION



Landesärztekammer Baden-Württemberg • Postfach 700361 • 70573 Stuttgart

02.11.2016

Universität Hohenheim  
Institut für Ernährungsmedizin (180c)  
Frau Prof. Dr. Anja Bosy-Westphal  
Fruwirthstraße 12  
70599 Stuttgart

Ansprechpartnerin:  
fuesun.koc@laek-bw.de  
Tel.: 0711 76989-60  
Fax: 0711 76989-856

**Internes Aktenzeichen:** F-2016-099  
**Titel:** Einfluss des Energy Flux auf die kurzfristige Regulation der Energie- und Makronährstoffbilanz

**Antrag vom:** 07.10.2016

Sehr geehrte Frau Prof. Bosy-Westphal,

die Studie hat der Ethik-Kommission in ihrer Sitzung am 25.10.2016 zur berufsrechtlichen Beratung vorgelegen.

Von Seiten der Ethik-Kommission bestehen keine Bedenken.

#### Allgemeine Hinweise:

- Sämtliche Dokumente sind mit Versionsnummer und Versionsdatum zu versehen, um geänderte Dokumente voneinander unterscheiden zu können. Der Prüfplan ist zu unterschreiben (samt Datum, zu welchem unterschrieben wurde); Gleiches gilt für Amendments.
- Wir möchten Sie außerdem bitten, für eine raschere Bearbeitung bei zukünftigen Einreichungen jede einzelne Änderung im Text deutlich zu markieren (gestrichener Text wird durchgestrichen, ergänzter Text wird unterstrichen).

Unabhängig vom Beratungsergebnis macht die Ethik-Kommission darauf aufmerksam, dass die ethische und rechtliche Verantwortung für die Durchführung einer Studie beim Studienleiter und bei allen teilnehmenden Ärzten liegt.

Die Ethik-Kommission der Landesärztekammer Baden-Württemberg setzt sich zusammen und arbeitet gemäß den nationalen gesetzlichen Bestimmungen und der ICH-GCP-Leitlinie. Den Beratungen der Ethik-Kommission der Landesärztekammer Baden-Württemberg liegt die Deklaration des Weltärztebundes von Helsinki zugrunde.

Wir möchten darauf hinweisen, dass die Stellungnahme der Ethik-Kommission und die studienrelevante Korrespondenz an alle teilnehmenden Ärzte weiterzuleiten ist.

Bitte teilen Sie uns das Ende der Studie mit und senden Sie uns eine Synopsis des Abschlussberichts.

**LANDESÄRZTEKAMMER BADEN-WÜRTTEMBERG**

KÖRPERSCHAFT DES ÖFFENTLICHEN RECHTS

**ETHIK-KOMMISSION**



Mit freundlichen Grüßen

Dr. med. G. Hook

Stellvertretender Vorsitzender der Ethik-Kommission

**Eingereichte Unterlagen:**

**Mit Schreiben vom 07.10.2016**

- Antragsformular vom 10.10.2016
- Prüfplan Version 1.0 vom 07.10.2016
- Probandeninformation und Einwilligungserklärung Version 1.0 vom 10.10.2016
- Rekrutierungsanzeige undatiert

<b>C   A   U</b>	Christian-Albrechts-Universität zu Kiel	Agrar- und Ernährungs- wissenschaftliche Fakultät
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### Declaration of co-authorship

If a dissertation is based on already published or submitted co-authored articles, a declaration from each of the authors regarding the part of the work done by the doctoral candidate must be enclosed when submitting the dissertation.




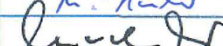
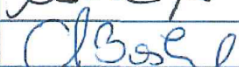
<b>1. Doctoral candidate</b>
<b>Name: Franziska Büsing</b>


<b>2. This co-author declaration applies to the following article:</b>
<b>Impact of energy turnover on the regulation of glucose homeostasis in healthy subjects</b>

The extent of the doctoral candidat's contribution to the article is assessed on the following scale:

- A. Has contributed to the work (0-33%)
- B. Has made a substantial contribution (34-66%)
- C. Did the majority of the work independently (67-100%)

3. Declaration on the individual phases of the scientific work (A,B,C)	Extent
<b>Concept:</b> Formulation of the basic scientific problem based on theoretical questions which require clarification, including a summery of the general questions which, it is assumed, will be answerable via analyses or concrete experiments/investigations	<b>A</b>
<b>Planning:</b> Planning of experiments/analyses and formulation of investigative methodology, including choice of method and independent methodological development, in such a way that the scientific questions asked can be expected to be answered	<b>B</b>
<b>Execution:</b> Involvement in the analysis or the concrete experiments/investgation	<b>C</b>
<b>Manuscript preparation: Presentation, interpretation and discussion of the results obtained in article form</b>	<b>C</b>

4. Signature of all co-authors		
Date	Name	Signature
04.04.19	Franziska Anna Hägele	
10.04.19	Alessa Nas	
24.04.19	Mario Hasler	
06.04.19	Manfred James Müller	
4.04.19	Anja Bosy-Westphal	

5. Signature of doctoral candidate		
Date	Name	Signature
03.04.2019	Franziska Büsing	

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**Franziska Büsing**

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**CURRICULUM VITAE**

21.08.1990 geboren in Karlsruhe

Staatsbürgerschaft deutsch

---

**Akademischer Werdegang**

---

- 2018-2019 **Fortsetzung der Dissertation zum Dr. oec. troph.**  
Titel der Dissertation: Impact of glycemia and energy balance on metabolic risk in healthy subjects  
Betreuerin: Frau Prof. Dr. oec. troph. Dr. med. Anja Bosy-Westphal
- 2016-2018 **Beginn einer Dissertation unter Betreuung von Frau Prof. Dr. oec. troph. Dr. med. Anja Bosy-Westphal**  
Universität Hohenheim am Institut für Ernährungsmedizin, Fachgebiet angewandte Ernährungswissenschaft und Diätetik
- 2014 – 2016 **Masterstudium der Ernährungsmedizin (M.Sc.)**  
Universität Hohenheim  
Titel der Masterarbeit: *Untersuchungen der intestinalen Barriere in einem entzündlichen Maus-Modell mit einer spezifischen Deletion des Caspase-8 Gens in den intestinalen Epithelzellen (Casp8<sup>ΔIEC</sup>) nach einer vierwöchigen diätetischen Intervention mit Inulin, Butyrat und Fructose*
- 2011 – 2014 **Bachelorstudium der Ernährungswissenschaft (B.Sc.)**  
Friedrich-Schiller-Universität Jena (2011-2012) und Universität Hohenheim (2012-2014)  
Titel der Bachelorarbeit: *Analyse zum Wissensstand der Verbraucher und Fachkreise zu den Themen „Probiotika“ und „Darmflora“*
- 2001 – 2010 **Abitur**  
St. Dominikus Gymnasium in Karlsruhe

## Originalarbeiten

---

Impact of energy turnover on fat balance in healthy adults during energy balance, caloric restriction and overfeeding; submitted for publication in British Journal of Nutrition; 04/2019.

Nas A, **Büsing F**, Hägele FA, Hasler M, Müller MJ, Bosy-Westphal A

Impact of energy turnover on the regulation of glucose homeostasis in healthy subjects; submitted for publication in Diabetes & Nutrition; 03/2019.

**Büsing F**, Hägele FA, Nas A, Hasler M, Müller MJ, Bosy-Westphal A

A high energy turnover improves appetite control at different levels of energy balance; submitted for publication in Cell Metabolism; 03/2019.

Hägele FA, **Büsing F**, Nas A, Hasler M, Müller MJ, Blundell JE, Bosy-Westphal A

High intake of orange juice and cola differently affects metabolic risk in healthy subjects; Clin Nutr. 2019 Apr;38(2):812-819. doi: 10.1016/j.clnu.2018.02.028. Epub 2018 Mar 3.

**Büsing F**, Hägele FA, Nas A, Döbert LV, Fricker A, Dörner E, Podlesny S, Aschoff J, Pöhl T, Schweiggert R, Fricke WF, Carle R, Bosy-Westphal A

High orange juice consumption with or in-between three meals a day differently affects energy balance in healthy subjects; Nutr Diabetes. 2018 Apr 25;8(1):19. doi: 10.1038/s41387-018-0031-3.

Hägele FA, **Büsing F**, Nas A, Aschoff J, Gnädinger L, Schweiggert R, Carle R, Bosy-Westphal A

## Kongress- und Tagungsbeiträge (1)

---

**Büsing F**; Vergleich von regelmäßigem Orangensaft- vs. Cola-Konsum im Hinblick auf den Harnsäurespiegel und die Regulation der Glykämie; 21.11.2018; Life Science Student Conference Kiel 2018 – Posterpräsentation

**Büsing F**, Hägele FA, Nas A, Bosy-Westphal A; Impact of energy flux on changes in glucose metabolism with caloric restriction and overfeeding; 09.-10.Nov 2018 Diabetes Herbsttagung / Jahrestagung der Deutschen Adipositas-Gesellschaft 2018, Wiesbaden, Deutschland – Posterpräsentation

## Kongress- und Tagungsbeiträge (2)

---

**Büsing F**, Hägele FA, Nas A, Bosity-Westphal A; Impact of energy flux on changes in glucose metabolism with caloric restriction and overfeeding; 09.-10.Nov 2018 Diabetes Herbsttagung / Jahrestagung der Deutschen Adipositas-Gesellschaft 2018, Wiesbaden, Deutschland – Vortrag

**Büsing F**; Ernährungstherapie zur Prävention und Behandlung diabetischer Stoffwechselstörungen; 17.August 2018; Intensivkurs Diabetologie, DKD Wiesbaden, Deutschland – Vortrag

**Büsing F**, Hägele FA, Nas A, Hummel J, Bosity-Westphal A; Impact of energy flux on changes in glucose metabolism with caloric restriction and overfeeding; 25.-27.Juni 2018; 11th International Symposium on In-Vivo Body Composition Studies, New York, USA – Posterpräsentation

**Büsing F**, Hägele FA, Nas A, Hummel J, Bosity-Westphal A; Impact of energy flux on changes in glucose metabolism with caloric restriction and overfeeding; 21.-23.Juni 2018; Ernährung 2018, DGEM, Kassel, Deutschland – Vortrag

**Büsing F**, Hägele FA, Nas A, Hummel J, Bosity-Westphal A; Impact of physical activity on daylong glycemia and insulin sensitivity during equal energy balance, caloric restriction and overfeeding; 07.- 09. März 2018; 55. DGE Kongress, Hohenheim, Deutschland – Vortrag

**Büsing F**, Hägele FA, Nas A, Bosity-Westphal A; Impact of physical activity on regulation of blood pressure during energy balance, overfeeding and caloric restriction; 20.-22.Okt 2017; 4th international conference on: Recent Advances and Controversies in the Measurement of Energy Metabolism (RACMEM), Fribourg, Schweiz – Posterpräsentation

**Büsing F**, Hägele FA, Nas A, Kaelble A, Aschoff J, Carle R, Bosity-Westphal A; Impact of regular orange juice or cola consumption on uric acid levels in healthy adults; 17.-20.Mai 2017; ECO (24th European Congress on Obesity), Porto, Portugal – Posterpräsentation

**Büsing F**; Hägele FA, Nas A, Aschoff J, Gnädinger L, Carle R, Bosity-Westphal A; Vergleich von regelmäßigem Orangensaft- vs. Cola-Konsum im Hinblick auf den Harnsäurespiegel und die Regulation der Glykämie; 01.-03.März 2017; 54. DGE-Kongress, Kiel, Deutschland – Vortrag

**Büsing F**; Hägele FA, Nas A, Aschoff J, Gnädinger L, Carle R, Bosity-Westphal A; Regulation des Blutzuckerspiegels bei Orangensaftkonsum zu und zwischen den Mahlzeiten; 01.-03.März 2017; 54. DGE-Kongress, Kiel, Deutschland – Posterpräsentation

## Stipendien und Auszeichnungen

---

- |      |  |
|------|--|
| 2019 | <b>Reisestipendium</b> der Deutschen Diabetes Gesellschaft, 54. Diabetes Kongress 2019 in Berlin                     |
| 2018 | <b>Reisestipendium</b> der Deutschen Diabetes Gesellschaft, Vortragsreise zur 12. DDG Herbsttagung 2018 in Wiesbaden |
| 2018 | <b>Posterpreis</b> , Kongress Ernährung 2018 in Kassel   |
| 2018 | <b>Forschungsförderpreis</b> der Baumann-Gonser Stiftung   |

---

Ort und Datum

---

Unterschrift

## **Danksagung**

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