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Impact of meal frequency and energy turnover on the regulation of energy intake

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Wer aufhört besser werden zu wollen, hört auf gut zu sein.

Marie von Ebner-Eschenbach

Meinen Eltern Susanne und Reimund

Vorbemerkung

Die vorliegende Dissertation wurde in kumulativer Form angefertigt und beruht auf zwei veröffentlichten Manuskripten. Zusätzlich zur fortlaufenden Seitennummerierung der Arbeit sind die Seitenzahlen der publizierten Manuskripte abgedruckt. Das Inhaltsverzeichnis bezieht sich auf die fortlaufende Seitennummerierung der Arbeit.

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List of abbreviations

AgRP	Agouti-related protein
AMPK	AMP-activated protein kinase
BMI	body mass index
CCK	cholecystokinin
DGE	Deutsche Gesellschaft für Ernährung e.V.
DPP-IV	dipeptidyl peptidase-IV
EI	energy intake
ET	energy turnover
FM	fat mass
GHS-R	growth-hormone-secretagogue receptor
GLP-1	glucagon-like peptide-1
IL-6	interleukin-6
MDC	minimal detectable change
NPY	neuropeptide Y
PAL	physical activity level
POMC	proopiomelanocortin
PYY	peptide YY
SEM	standard error of mean
SD	standard deviation
SSB	sugar sweetened beverage
tAUC	total area under the curve
VO _{2max}	maximal oxygen uptake
WHO	World Health Organization

Chapter I:

General Introduction

Body weight control is basically understood in an energy balance framework with equal energy intake and energy expenditure being required for maintaining a stable body weight. According to the first law of thermodynamics energy can be transformed but not created or destroyed, which is why changes in energy intake and/or expenditure lead to changes in energy stores and therefore changes in body composition and weight (Hall *et al.*, 2012). A chronically positive energy balance is therefore commonly seen as reason for weight gain and obesity (Williams & Wu, 2016). Generally, there are some homeostatic mechanisms to keep an equal energy balance but this regulation is not controlled very tightly in the short-term as energy balance varies markedly within one day between fasting and postprandial periods (Hall *et al.*, 2012) as well as between several days. For example, during weekends or holidays energy balance becomes positive due to enhanced energy intake leading to so called *weekend* or *holiday weight gain* which is at least partly compensated for during the following working days (Racette *et al.*, 2008; Schoeller, 2014). Approximate equal energy balance is therefore thought to be attained over longer periods, e.g. over one week (Edholm, 1977). Nevertheless, if

such short periods of overfeeding occur regularly they can result in gradual weight gain if they are inadequately compensated e.g. by a higher energy expenditure.

Obesity prevention is more than eating less and exercising more. More genes known to be involved in obesity predisposition modulate energy intake than energy expenditure (Pérusse *et al.*, 2005). Consequently, energy intake seems to be more crucial for energy balance and therefore to prevent obesity than energy expenditure. Consistently, it has been shown that the effect of exercise on energy expenditure is commonly exaggerated (Zelasko, 1995). Therefore, it is not enough to take both sides of the energy balance equation into account since the underlying mechanisms include factors like appetite control and body composition (Manore *et al.*, 2017). In fact, long- and short-term appetite control have an important impact on energy balance and appetite control itself is affected by many factors as for example energy intake can be increased by stress (Epel *et al.*, 2001) and by sleep deprivation or sleep deficit (Spiegel *et al.*, 2004). Meal timing also has an influence on body weight control since hunger follows a circadian rhythm and is highest in the evening (Scheer *et al.*, 2013). Consistently, it has been shown that the same energy consumed later in the day impedes weight loss (Garaulet *et al.*, 2013). Meal frequency also seems to have an impact on appetite control but it remains unclear to which extent the frequency of energy containing beverage consumption has an effect. Appetite control may also be influenced by the level of energy balance which is described as energy turnover but this effect remains to be demonstrated under laboratory controlled conditions. A better understanding especially of the impact of meal frequency and energy turnover on appetite control may lead to public health recommendations for the prevention of weight gain and for weight maintenance. Therefore, this thesis focuses on the impact of meal frequency and energy turnover on energy intake which both are explained in more detail following a paragraph on appetite control in general.

Appetite control

The system of appetite control is complex and includes endocrine and afferent signals. The center of appetite control is located in the arcuate nucleus and the paraventricular nucleus, the energy intake regulating area in the hypothalamus (for review see (Kalra *et al.*, 1999)). Here all peripheral signals arrive, are integrated and result either in enhanced or in decreased energy intake. Incoming signals can be distinguished in tonic and episodic signals. Tonic signals from the periphery indicate the body's energy stores mediated by leptin and insulin (Woods *et al.*, 1974). In the arcuate nucleus, these hormones lead to inhibition of energy intake via enhanced secretion from anorexigenic proopiomelanocortin (POMC) neurons and inhibited secretion from orexigenic neuropeptide Y (NPY)- and agouti-related protein (AgRP)-producing neurons (for review see (Woods *et al.*, 2006)). In addition, the energy expenditure of metabolically active tissues provide constant signals to increase energy intake (Javed *et al.*, 2010). These tonic signals are relatively invariable and show no greater fluctuations between or within days. In contrast, episodic signals from the gastrointestinal tract vary widely during days and affect timing, type and amount of the food consumed. These episodic signals are mediated by peptide hormones secreted in the gastrointestinal tract and can be separated in two groups: the anorexigenic peptides like glucagon-like peptide-1 (GLP-1), peptide YY (PYY) and cholecystokinin (CCK) and the orexigenic peptide ghrelin (Murphy & Bloom, 2006).

GLP-1, a 30 amino acid peptide was discovered in the 1980s in the lab of Dr. Habener (Lund, 2005). It is mainly secreted after meals from L-cells in the distal small intestine as GLP-1_{7-36amid} and promotes satiety while reducing energy intake (Flint *et al.*, 1998). The active form is rapidly degraded by the enzyme dipeptidyl peptidase-IV (DPP-IV) located in the enterocyte brush border and in the endothelial cells lining the capillaries of the lamina propria. Therefore, a large part of the secreted peptide is leaving the gut already in the inactivated form (Hansen *et al.*, 1999). Further rapid degradation caused by DPP-IV takes place on whole body level resulting in an half-life of 1-2 min of intact GLP-1 in plasma (Vilsbøll *et al.*, 2003). Thus, it is unlikely that GLP-1_{7-36amid} has an endocrine effect on the arcuate nucleus. Instead, neuroendocrine signals are mediated via vagal sensory

afferents to the solitary tract nucleus in the brain stem since GLP-1 receptors were found in afferent fibers located in the gut and passed by GLP-1 on its way to the capillary (Nakagawa *et al.*, 2004). The signals arriving in the solitary tract nucleus are then passed on to the arcuate nucleus and the paraventricular nucleus in the hypothalamus where they, together with endocrine but minor input from circulating GLP-1, stimulate secretion from POMC-neurons and inhibit secretion from NPY- and AgRP-producing neurons leading to reduced energy intake (Tungland, 2018).

Ghrelin, a peptide of 28 amino acids was discovered in 1999 by Kojima *et al.* (Kojima *et al.*, 1999). It is mainly secreted from X/A-like cells in the stomach with concentrations rising during fasting periods until meal initiation whereas levels fall afterwards proportional to the ingested energy content (Callahan *et al.*, 2004; Cummings *et al.*, 2004). Ghrelin promotes subjective feelings of hunger and is therefore often referred to as the 'hunger hormone' (Kojima & Kangawa, 2005). Acylated ghrelin with an additional *n*-octanoyl group at serine three is the active form which is able to cross the blood-brain barrier and to bind to the growth-hormone-secretagogue receptor (GHS-R). Ghrelin stimulates secretion from NPY- and AgRP-producing neurons and inhibits secretion of POMC-neurons leading to enhanced energy intake (Kojima & Kangawa, 2005). In rats, ghrelin receptors were also found on vagal afferent neurons suggesting that ghrelin signals from the stomach may be additionally mediated to the hypothalamus by the vagus nerve as neuroendocrine signals via the solitary tract nucleus (Date *et al.*, 2002).

Impact of meal frequency on energy intake

It is well known that eating patterns have changed towards an increasing consumption of snacks and thus an increasing meal frequency over the last decades (Kant & Graubard, 2015). This is in line with the association of a higher meal frequency and thus an equally distributed energy intake throughout the day with a reduced risk of obesity found in a subsamples of 3009 subjects in the Malmö Diet and Cancer cohort and of 499 subjects in the Seasonal Variation of Blood Cholesterol Study (Ma *et al.*, 2003; Holmbäck *et al.*, 2010). A high meal frequency is also recommended in cases of type 2 diabetes

mellitus and to prevent cardiovascular risk factors in the Scientific Statement of the American Heart Association from 2017 (St-Onge *et al.*, 2017). However, due to enhanced energy density of snacks a high meal frequency easily leads to a positive energy balance and weight gain (Zizza *et al.*, 2001). This is confirmed by the results of the Adventist Health Study 2 where after 7 years of follow-up in 50,660 adults it was found that the more meals and snacks consumed per day the larger is the relative increase in BMI per year showing a linear association (Kahleova *et al.*, 2017). Eating one or two meals/d resulted in a relative decrease of BMI per year of -0.05 kg/m^2 and -0.03 kg/m^2 compared to three meals/d. In contrast, eating four, five or \geq six meals/d resulted in a relative increase in BMI per year of $+0.02 \text{ kg/m}^2$, $+0.02 \text{ kg/m}^2$ and $+0.04 \text{ kg/m}^2$, respectively compared to three meals/d (Kahleova *et al.*, 2017).

Focusing on the impact of meal frequency on appetite control, it was shown that eating 3 meals/d reduces hunger and increases satiety due to a trend of reduced ghrelin and increased GLP-1 levels compared to an isocaloric energy intake with 14 meals/d (Munsters & Saris, 2012). These results were confirmed by the finding that 6 meals/d lead to increased hunger and desire to eat compared to 3 meals/d (Ohkawara *et al.*, 2013). Likewise, after three weeks of 8 vs. 3 meals/d an acute meal test with 2 vs. 1 isocaloric meals within four hours resulted in increased desire to eat and overall appetite with high meal frequency (Perrigue *et al.*, 2016). However, decreasing meal frequency from 3 to 2 isocaloric meals/d led to decreased satiety (Smeets & Westerterp-Plantenga, 2008). And a decrease from 3 to 1 meals/d showed increased hunger and desire to eat even when the meal frequency lasted for 8 weeks (Stote *et al.*, 2007). These findings were confirmed in a symposium evaluating the impact of meal frequency on appetite control investigated in intervention studies concluding that increasing meal frequency above 3 meals/d has little to no effect whereas reducing meal frequency below 3 meals/d negatively effects appetite control (Leidy & Campbell, 2011). Therefore, a frequency of 3 meals/day seems to be favorable for an optimal appetite control and thus weight maintenance.

Today, increased snacking is not only due to the consumption of solid foods but also a great amount of sugar sweetened beverages (SSB) and other caloric beverages like e.g.

sweetened coffees, fruit drinks and fruit juices are consumed in-between meals (Zizza *et al.*, 2001). Since the sugar content of fruit juice is comparable to SSBs, not only a high consumption of SSBs but also a high intake of fruit juice and fruit drinks is associated with weight gain and obesity (Schulze *et al.*, 2004; Te Morenga *et al.*, 2012). But it remains questionable whether the timing of beverage consumption (with or in-between meals) and thus a variation in meal frequency has an impact on this outcome. In 14 healthy males, it has been shown that a 568 mL water preload immediately (<1 min) before a meal reduced hunger and subsequent energy intake in an *ad libitum* meal compared to no preload which was suggested to be mediated by gastric distension (Corney *et al.*, 2016). Incorporating water into a food preload also decreased hunger and subsequent energy intake in an *ad libitum* meal compared to drinking water with the preload or a preload without additional water as found in 24 healthy women (Rolls *et al.*, 1999). However, preloads of 590 mL energy containing beverages (orange juice, low-fat milk and regular cola) were not compensated for during the following *ad libitum* meal although they led to reduced hunger before the meal compared to 590 mL sparkling water in 32 healthy adults (Almiron-Roig & Drewnowski, 2003). Likewise, in 44 healthy women it has been shown that consuming 360 g of energy containing beverages (orange juice, low-fat milk, diet cola and regular cola) with the meal were not compensated for resulting in an enhanced total energy intake compared to water (DellaValle *et al.*, 2005). These results indicate that regardless whether the energy containing beverage is consumed before or with the meal, the following food consumption will not be adequately reduced for the already ingested energy content resulting in an enhanced total energy intake compared to water preload or no beverage (Almiron-Roig & Drewnowski, 2003; DellaValle *et al.*, 2005). In contrast, a preload of whole fruit apple resulted in enhanced satiety and reduced energy intake in an *ad libitum* lunch compared to preloads of applesauce and apple juice (Flood-Obbagy & Rolls, 2009; Mattes & Campbell, 2009). Even if naturally occurring levels of fiber were added to the juice satiety was not increased (Flood-Obbagy & Rolls, 2009). A possible explanation besides the fact that high caloric snacks by themselves contribute to an increased energy intake is therefore that apparently 'liquid calories' are not as satiating as 'solid calories' (Flood-Obbagy & Rolls, 2009; Mattes & Campbell, 2009). Therefore, the impact of an increased meal

frequency due to the consumption of a considerable amount of orange juice in-between meals compared to with meals (reduced meal frequency with higher meal volume) on appetite control should be examined in long-term free-living interventions to verify the effects observed in acute meal tests.

Impact of energy turnover on energy intake and appetite control

Appetite and energy intake are supposed to be asymmetrically controlled: An energy deficit is actively compensated for whereas overfeeding seems to be tolerated (Müller *et al.*, 2015). On the other hand, energy intake seems to match energy expenditure at high levels of physical activity whereas intake far exceeds expenditure at low levels of physical activity (Mayer *et al.*, 1956). This assumption is based on the observations from Jean Mayer more than 50 years ago where he described mill workers with an increasing level of physical work meeting their needs by a corresponding increase in energy intake. But workers with low physical work and increasing sedentariness showed an increasing energy intake as well (Mayer *et al.*, 1956). To verify and to further examine this observation of an asymmetric appetite control the energy turnover approach is highly suitable. An equal energy balance can be achieved by a low energy turnover where energy expenditure and the corresponding energy intake are both low or by a high energy turnover where expenditure and intake are both high. Therefore, an increase in energy requirement with enhanced physical activity is taken into account by the energy turnover approach which is mandatory to investigate appetite control independent from energy balance.

In a metabolic chamber study, six lean men were each studied twice for 7 days during *ad libitum* energy intake while being sedentary (physical activity level (PAL) 1.4) and being active (PAL 1.8). This decrease of the PAL did not provoke a reduction in energy intake resulting in a positive energy balance and weight gain with the sedentary regime. Since a positive fat balance was measured the weight gain seems to be primarily due to a gain in fat mass (Stubbs *et al.*, 2004). In another cross-over trial 14 healthy adults completed three 24h-interventions: one active day (pursued PAL 2.05, obtained by low

intensity activities), one inactive day (pursued PAL 1.39) and one inactive day with overfeeding (pursued PAL 1.39). During the active and inactive day subjects were fed in approximate energy balance, during the inactive day with overfeeding subjects were fed the same diet as on the active day. It was shown that the decrease in energy expenditure did not result in correspondingly reduced appetite suggesting that inactivity may promote a positive energy balance leading to weight gain (Granados *et al.*, 2012). In an observational trial in 421 subjects over 12 months it was found that at low levels of habitual physical activity (less than 7116 steps/day) energy intake is not matched to energy expenditure but is associated with fat mass gain over one year (Shook *et al.*, 2015). In a pilot study 6 obese adults were investigated twice for 5 days of low energy turnover (PAL 1.35) and high energy turnover (PAL 1.7) after weight loss and subsequent weight stabilization under free-living conditions (Paris *et al.*, 2016). A high energy turnover resulted in reduced hunger seeming to decrease the biologic drive to eat and thus may be superior in maintaining weight loss compared to low energy turnover. However, this effect was only seen with an acute high energy turnover and was no longer evident in the following morning (Paris *et al.*, 2016). Although the impact of energy turnover is only investigated in short-term interventions so far it is assumed that maintaining a high energy turnover is a successful strategy to prevent weight (re-) gain (Hand *et al.*, 2015). This assumption seems to be confirmed since it was shown that habitual exercisers can better compensate for preload manipulations due to an increased sensitivity of short-term food intake compared to non-exercisers (Long *et al.*, 2002; Van Walleghe *et al.*, 2007; Beaulieu *et al.*, 2017). A possible mechanism is the phenomenon of so called 'exercise-induced anorexia' (King *et al.*, 1994) which is discussed to be mediated by a reduction in ghrelin and an increase in GLP-1 and PYY after an acute bout of exercise (Stensel, 2010). However, it is important that direct comparisons between different levels of energy turnover can only be made at a constant energy balance. To achieve a constant energy balance during different levels of energy turnover the exact amount of energy expended needs to be known and the exact same amount of energy needs to be consumed. This can be realized in the complex setting of a metabolic chamber where 24h-energy expenditure is measured and the exact amounts of weighed food is provided. However, a complete validation of the Mayer-Hypothesis in

a highly controlled and randomized intervention to reveal the underlying mechanisms is still lacking.

Objectives

This thesis investigates the impact of meal frequency and energy turnover on the regulation of energy intake in healthy adults and the following objectives were addressed:

- (i) The first objective was to examine the impact of varying meal frequency due to orange juice consumption with or in-between meals on energy intake and during a free-living nutritional intervention (Chapter II)
- (ii) The second objective was to examine the impact of energy turnover on appetite control at different energy balances in a controlled intervention in a metabolic chamber (Chapter III)

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Chapter II:

High orange juice consumption with or in-between three meals a day differently affects energy balance in healthy subjects

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ARTICLE

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High orange juice consumption with or in-between three meals a day differently affects energy balance in healthy subjects

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Abstract

Sugar-containing beverages like orange juice can be a risk factor for obesity and type 2 diabetes although the underlying mechanisms are less clear. We aimed to investigate if intake of orange juice with or in-between meals differently affects energy balance or metabolic risk. Twenty-six healthy adults (24.7 ± 3.2 y; BMI 23.2 ± 3.2 kg/m²) participated in a 4-week cross-over intervention and consumed orange juice (20% of energy requirement) either together with 3 meals/d (WM) or in-between 3 meals/d (BM) at ad libitum energy intake. Basal and postprandial insulin sensitivity (primary outcome), daylong glycaemia, glucose variability and insulin secretion were assessed. Body fat mass was measured by air-displacement plethysmography. After BM-intervention, fat mass increased ($+1.0 \pm 1.8$ kg; $p < 0.05$) and postprandial insulin sensitivity tended to decrease (Δ Matsuda_{ISI}: -0.89 ± 2.3 ; $p = 0.06$). By contrast, after WM-intervention fat mass and gamma-glutamyl transferase (GGT) decreased (-0.30 ± 0.65 kg; -2.50 ± 3.94 ; both $p < 0.05$), whereas glucose variability was higher (Δ MAGE: $+0.45 \pm 0.59$, $p < 0.05$). Daylong glycaemia, insulin secretion, changes in basal insulin sensitivity, and triglycerides did not differ between WM- and BM-interventions (all $p > 0.05$). In young healthy adults, a conventional 3-meal structure with orange juice consumed together with meals had a favorable impact on energy balance, whereas juice consumption in-between meals may contribute to a gain in body fat and adverse metabolic effects.

Introduction

A high intake of sugar-containing beverages is associated with overweight and obesity¹ and is therefore suspected to promote weight gain. Increased consumption of fruit juice was indeed associated with 4-year weight gain similar to increased intake of fruit punch². Since 100% fruit juice has a comparable sugar-content to sugar-sweetened beverages (SSB), the consumption of fruit juice may also be associated with an increased risk for type 2 diabetes³. Fruit juices contain important

nutrients (i.e., vitamin C, potassium, folate, magnesium, and β -carotene⁴) and flavonoids, and are an important contributor to total fruit intake⁵. Whereas orange juice has long been an integral part of a traditional (continental and American) breakfast, soft drinks are usually consumed to satisfy one's thirst. Consequently, the effect of orange juice consumption with meals versus in-between meal ingestion should be investigated regarding the effect of energy balance and metabolic risk.

Several mechanisms have been proposed to explain the association between consumption of sugar-containing beverages and weight gain. First, it has been shown that the consumption of energy-dense beverages does not lead to a lower food intake in an acute meal setting, thus leading to a higher total energy intake^{6–9}. This is

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also true for observational studies where the energy consumed from caloric beverages was not compensated by reducing solid food intake^{10, 11}. These findings were explained by a low satiety value of beverages (for review see¹²). Fruit juice contains very little or no fiber, and has been shown to be less satiating than whole fruits¹³. In line with this finding, in contrast to whole fruits, consumption of fruit juices has been shown to promote long term weight gain¹⁴. The second proposed mechanism is known as the “carbohydrate-insulin theory of obesity” and is based on the high glycemic index of sugar-containing drinks, leading to postprandial hyperinsulinemia and thus promoting fat storage and inhibiting fat oxidation^{15, 16}. The rapid decline in blood glucose levels caused by postprandial hyperinsulinemia is also believed to increase appetite and thus subsequent energy intake¹⁶. Both effects, remain however insufficiently substantiated by scientific evidence and thus are highly controversial (for review see^{17, 18}).

Intake of sugar-containing drinks with or in-between meals may differently affect metabolic regulation, because postprandial glycemia is lowered especially by protein content of the diet, which is due to slower gastric emptying and enhanced insulin response¹⁹. On the other hand, intake of juice in-between main meals (e.g., snacking behavior) may prevent the drop in insulin between meals and thus may inhibit effective lipid oxidation. According to the “carbohydrate-insulin theory of obesity” fasting periods in-between meals are important, because the decrease in insulin levels activates lipolysis and lipid oxidation which may improve fat balance¹⁵. In addition, increased meal frequency is

suggested to be a risk factor for increased energy intake²⁰. We therefore hypothesized that in-between meal consumption of fruit juice leads to a positive energy balance and increases metabolic risk, whereas intake of juice together with three main meals can prevent these adverse effects.

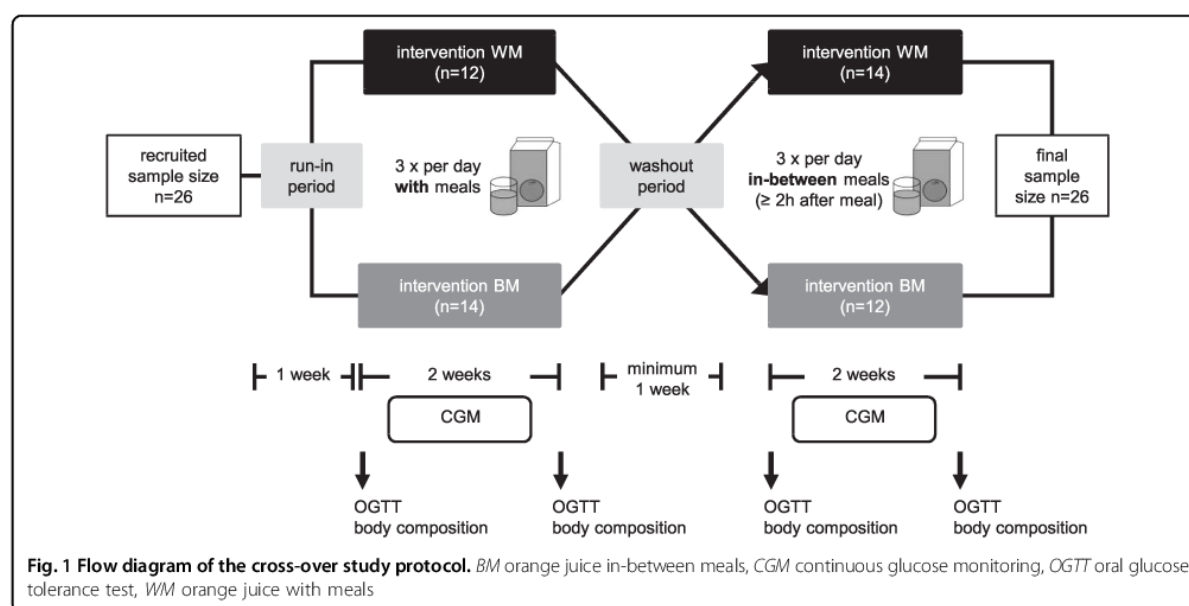
Methods

Study population

Twenty-six healthy adults (13 women, 13 men) aged between 20 and 45 years were recruited in April and September 2016 by notice board postings at the Universities of Hohenheim and Stuttgart. Exclusion criteria were daily consumption of fruit juice or SSB, fructose intolerance, habitual meal skipping, chronic diseases, regular use of medication or supplements, alternative eating habits and smoking. The study protocol was approved by the ethics committee of the State Medical Council of Baden-Württemberg, Germany. This trial was registered at clinicaltrials.gov under NCT02974478. All subjects provided written informed consent before participation. In a previous study we observed a 17% difference in postprandial insulin sensitivity after consumption of high vs. low GI-SSBs²¹. Twenty-five participants are therefore needed to detect a difference in Matsuda-index of similar magnitude between orange juice consumption with and in-between meals (assuming a power of 80% and an alpha level of 5%).

Study protocol

A cross-over free-living nutrition intervention was conducted at the Institute of Nutritional Medicine,



University of Hohenheim in Stuttgart, Germany. An outline of the study protocol is given in Fig. 1. The primary outcome was insulin sensitivity and the secondary outcome body fat mass. Prior to the intervention, subjects kept a nutrition diary to assess normal nutrition habits and were asked to maintain their habitual choice of foods throughout the study. During a 1 week run-in period and the subsequent entire intervention, subjects were instructed to avoid the consumption of citrus fruits, additional orange juice and SSB in order to obtain equal baseline conditions and avoid confounders. A minimum of 1 week washout period was used between the 2-week consumption of each intervention period. Participants in the first group recruited in April began with the with meal (WM)-intervention, while participants in the second group recruited in September began with the in-between meal (BM)-intervention. Throughout intervention periods, participants were asked to consume only three meals a day and drink orange juice three times a day either with meals (WM) or in-between meals (BM) for two weeks. During the BM-intervention, participants were instructed to drink the orange juice not less than 2 h after meals. Participants came to the institute for visits at the beginning (day 1) and end (day 15) of each intervention phase. On these four visiting days (T1–T4) body composition was measured and an oral glucose tolerance test was performed. Subjects were asked to go to bed before midnight and to abstain from vigorous activity and alcohol consumption prior to visiting days to avoid potential bias on glucose tolerance.

Intervention with orange juice

The orange juice was 100% juice with pulp, containing 43 kcal/100 mL and 8.9 g sugar (3.2 g sucrose, 2.7 g glucose and 3.0 g fructose)/100 mL according to the manufacturer and as verified by own analyses. The amount of orange juice provided was defined as 20% of individual energy requirement. Energy requirement was assessed by multiplying resting energy expenditure (REE, predicted according to²²) by physical activity level (PAL). Subjects were interviewed about their habitual daily activity and an individual PAL between 1.4 (low active) and 1.8 (active) was estimated²³. The individual amount of orange juice was calculated at the beginning of each intervention phase to meet 20% of daily energy requirement. The amount of orange juice per serving was weighted in bottles and the filling line was marked. For each serving, subjects refilled the bottles up to the marking. All orange juice was provided by the Institute of Nutritional Medicine.

Fasting and postprandial glucose metabolism

On visiting days, subjects came to the Institute between 6:30 a.m. and 8:00 a.m. Blood samples were collected after an overnight fast (≥ 10 h) and 30, 60, and 120 min after a

standard oral glucose tolerance test (OGTT, intake of 75 g glucose, Accu-Chek® Dextrose O.G-T., Roche Diagnostics GmbH, Mannheim, Germany). Basal insulin sensitivity was calculated using Homeostatic Model Assessment-insulin resistance (HOMA-IR = fasting glucose (mg/dl) x fasting insulin (μ U/mL) /405;²⁴), which is a valid index for the assessment of hepatic insulin sensitivity²⁵. Postprandial insulin sensitivity was assessed by Matsuda Insulin Sensitivity Index (Matsuda_{ISI} = 10,000/ $\sqrt{(\text{fasting glucose} \times \text{fasting insulin}) \times (\text{mean glucose} \times \text{mean insulin during OGTT})}$), which is a validated index and was developed especially for the assessment of insulin sensitivity using OGTT-data²⁶. Interstitial glucose concentrations were measured by continuous glucose monitoring (CGM, Dexcom G4 Platinum, Nintamed GmbH & Co KG, Mainz, Germany) for 7 days during each intervention. The sensor was placed at the back of the upper arm to monitor glucose levels in the subcutaneous tissue. Sensor readings were reported every 5 min. CGM-devices were calibrated twice a day against fasting capillary blood samples. Area under the curve (AUC) was calculated as incremental AUC (iAUC) for 18 h (6:00–00:00 a.m.) from 3–5 valid daylong CGM-data sets using trapezoidal rule²⁷. Daylong glucose variability was determined by mean amplitude of glycemic excursions (MAGE) using 24-h CGM-data:

$$\sum_{\chi}^{\lambda} \text{ with } \lambda > \gamma$$

where λ is the difference from peak to nadir, χ is the number of valid observations, and γ is 1 SD of mean interstitial glucose values in a 24-h period^{28, 29} using a published macro³⁰. Fasting fructosamine was determined to assess average glycemia during the preceding 1–3 weeks³¹. Daylong insulin secretion was assessed by 24-h urinary C-peptide excretion at the end of each intervention phase.

Blood sampling and analytical methods

Blood sampling was conducted by vein cannula. Plasma glucose was determined using hexokinase method, and serum insulin and urinary C-peptide excretion were measured by electrochemiluminescence immunoassay. Serum gamma-glutamyl transferase (GGT), triglycerides (TG) and serum fructosamine were measured by photometry before and after each intervention phase, respectively.

Body composition

Height was measured using a stadiometer (seca 274, seca GmbH & Co.KG, Hamburg, Germany). Body composition was assessed from the mean of duplicate measurements using air displacement plethysmography (ADP) by the BodPod™ Body Composition System (COSMED USA, Inc., Concord, CA, USA) at the beginning and end of each intervention phase. ADP is based on the assessment of body density from weight (Tanita scale

coupled to the BodPod™-System) and body volume and the subsequent calculation of percentage fat mass (FM) based on assumed constant densities of fat-free mass and FM. Minimal detectable change (MDC) of FM measured by ADP (BodPod™ device) was 0.53 kg as assessed from duplicate measurements in 7 subjects (BMI: 19.6–25.0 kg/m²; FMI: 3.9–7.2 kg/m²). Fat mass index (FMI) was calculated as FM divided by height squared (kg/m²)³². Weight was assessed by an electronic scale coupled to the BodPod™ system.

Physical activity

Physical activity was continuously measured using a triaxial activity monitor (ActivPAL™, Paltechnologies Ltd., Glasgow, UK). The ActivPAL™ was fixed on the upper thigh with waterproof patches and was worn permanently during both intervention periods. Subjects were requested to maintain physical activity constant for the whole study period and to refrain from exercise one day prior visits.

Statistical analyses

Data analyses were performed using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA). Data are presented as mean ± SD. The assumption of normality was verified with the Kolmogorov–Smirnov test. Overall parameters HOMA-IR, GGT and TG as well as ΔFM and ΔHOMA-IR for BM-intervention did not meet the criteria of normal distribution. Differences between pre and post intervention (T1 vs. T2 and T3 vs. T4) as well as between changes with both interventions (ΔT2-T1 vs. ΔT4-T3) were analyzed by two-sided paired *t*-test or Wilcoxon-test, as appropriate. Differences in HOMA-IR and Matsuda_{ISI} between participants with a stable FM or a gain in FM, as well as differences between men and women were analyzed by Mann–Whitney-*U*-Test and independent *t*-test, respectively.

Relationships between normally distributed parameters were determined using Pearson correlation coefficients. Non-parametric relationships were analyzed using Spearman correlation coefficients. Power analysis was performed using the free-software G*Power 3.1.7. A *p*-value < 0.05 was considered to be statistically significant.

Results

Thirteen women and thirteen men aged 20–33 years (24.7 ± 3.2 years) and with a BMI between 19.1–33.3 kg/m² (23.2 ± 3.2 kg/m²) participated in this trial. According to WHO criteria five subjects were overweight and one participant was obese. The REE and therefore the intake of orange juice did not differ between both interventions (REE WM: 1662 ± 270 kcal/d vs. BM: 1663 ± 275 kcal/d; orange juice WM: 1277 ± 221 mL/d vs. BM: 1278 ± 224 mL/d; all *p* > 0.05). The amount of sugars provided by the

orange juice was 112.3 ± 19.4 g/d in WM-intervention and 112.5 ± 19.7 g/d in BM-intervention (difference not significant). During the entire BM-intervention, participants consumed 7697 ± 1349 kcal from orange juice which is equivalent to a theoretical gain in FM of 855 ± 150 g assuming an energy content of 9000 kcal/kg FM. Physical activity did not differ between both interventions (10,118 ± 3128 steps/d in WM vs. 10,528 ± 3438 steps/d in BM).

As shown in Table 1, FM significantly decreased after the intake of orange juice WM and increased after the BM-intervention (both, *p* < 0.05), whereas body weight did not change. During BM-intervention, 11 participants (5 women and 6 men) had an FM gain greater than the MDC (0.53 kg), whereby women gained more FM compared to men (+3.92 ± 2.06 vs. +1.30 ± 0.59 kg FM; *p* < 0.05). GGT also decreased with WM, whereas no changes were observed with BM-intervention. TG, postprandial and basal insulin sensitivity remained unchanged with both interventions. There was a tendency for a decrease in basal insulin sensitivity (*p* = 0.06) with ΔWM compared to ΔBM (Table 1). This is however likely explained by higher baseline levels in HOMA-index at WM-intervention compared to BM-intervention (*p* < 0.05), because higher baseline levels were associated with a larger decrease in HOMA-index due to WM-intervention (*r* = −0.62, *p* < 0.01). No differences in changes of Matsuda_{ISI} and HOMA-IR were observed between subjects with stable FM and a gain in FM after BM-intervention. During WM-intervention, there was a tendency for a positive correlation between ΔFM and ΔMatsuda_{ISI} (*r* = 0.38; *p* = 0.06), whereas this correlation was not observed with BM-intervention. MAGE-index and the maximum glucose value were significantly higher during WM-intervention when compared with BM-intervention, whereas iAUC glucose, C-peptide excretion (Table 2) and fructosamine (Table 1) did not differ between both interventions. Time course of daylong glycemia is shown in Fig. 2 for all participants (panel A) and illustrated for one exemplary participant (panel B) to demonstrate the higher glucose variability during WM-intervention as compared to BM-intervention. This variability is averaged out by the mean value from all subjects, because exact meal times were not prescribed by the study protocol. Although the BMI range in our population was 19.1–33.3 kg/m², there were no correlations between BMI and the changes in FM, Matsuda- and HOMA-index, GGT, iAUC glucose, MAGE-index and C-peptide excretion.

Discussion

In line with our hypothesis, the present study demonstrates that the impact of orange juice consumption on energy balance and metabolic risk depends on timing of juice intake in relation to meals. When compared with

Table 1 Comparison of energy balance and metabolic risk between interventions with orange juice with meals and in-between meals^a

	WM			BM			$\Delta\text{BM-WM}$ $\Delta\Delta(\text{T4-T3})-(\text{T1-T2})$
	T1	T2	$\Delta\text{T2-T1}$	T3	T4	$\Delta\text{T4-T3}$	
Energy balance							
weight, kg	70.3 ± 14.1	70.4 ± 14.4	+0.13 ± 0.82	70.1 ± 14.5	70.3 ± 14.5	+0.21 ± 0.94	NS
FM, kg	15.3 ± 6.8	15.0 ± 6.7	-0.30 ± 0.65*	14.1 ± 6.3	15.1 ± 6.6	+1.02 ± 1.81**	<i>p</i> = 0.001
FMI, kg/m ²	5.15 ± 2.3	5.05 ± 2.2	-0.10 ± 0.22*	4.78 ± 2.1	5.08 ± 2.2	+0.30 ± 0.58*	<i>p</i> = 0.019
Metabolic risk							
HOMA-IR	1.97 ± 1.1	1.79 ± 1.0	-0.18 ± 0.65	1.62 ± 0.8	1.63 ± 0.6	+0.01 ± 0.67	<i>p</i> = 0.062
Matsuda _{SI}	6.01 ± 2.7	6.08 ± 2.7	+0.07 ± 1.92	7.11 ± 2.9	6.21 ± 2.3	-0.89 ± 2.33	NS
Fructosamine, μmol/L	236.1 ± 25.3	234.0 ± 15.9	-2.0 ± 20.2	233.3 ± 13.0	236.0 ± 15.6	2.7 ± 15.7	NS
GGT, U/L	17.9 ± 7.9	15.4 ± 6.7	-2.50 ± 3.94**	16.9 ± 7.3	16.4 ± 6.6	-0.42 ± 4.49	NS
TG, mg/dL	100.9 ± 57.0	98.5 ± 47.7	-2.35 ± 45.83	87.9 ± 42.0	89.8 ± 34.8	+1.92 ± 33.80	NS

BM orange juice in-between meals, FM fat mass, FMI fat mass index, GGT gamma-glutamyl transferase, T1 study day before WM-intervention, T2 study day after WM-intervention, T3 study day before BM-intervention, T4 study day after BM-intervention, WM orange juice with meals

^aValues are means ± SDs, *n* = 26

p* < 0.05; *p* < 0.01, paired *t*-test or Wilcoxon-test

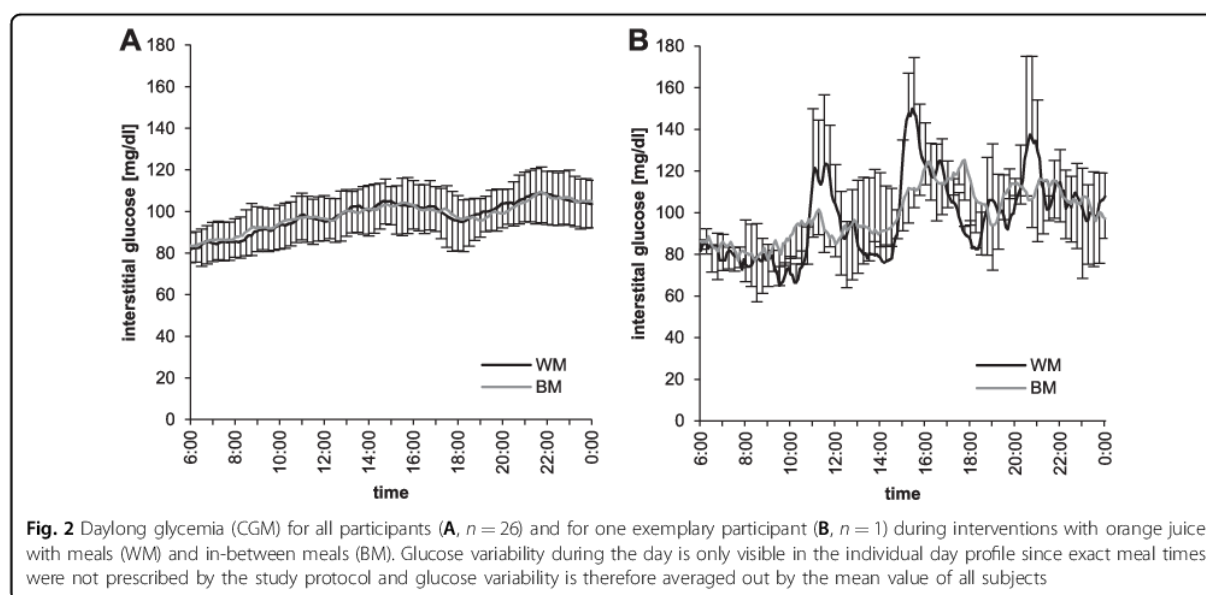
Table 2 Comparison of daylong glycemia and insulin secretion between interventions with orange juice with meals (WM) and in-between meals (BM)^a

	WM	BM	$\Delta\text{BM-WM}$
iAUC glucose, mg/dL x 18 h	289 ± 89	260 ± 72	-29.56 ± 93.55
MAGE	2.08 ± 0.3	2.52 ± 0.5	+0.45 ± 0.59**
MAX glucose, mg/dL	151 ± 12	143 ± 13	-7.88 ± 16.37*
C-peptide, μg/d	70.0 ± 37.0	70.6 ± 41.9	+0.52 ± 39.19

iAUC incremental area under the glucose curve for 18 h, MAGE mean amplitude of glycemic excursions, MAX glucose maximal interstitial glucose value per day

^aValues are means ± SD, *n* = 26

p* < 0.05, *p* < 0.01, paired *t*-test



in-between meal consumption, intake of orange juice together with breakfast, lunch and dinner prevented a positive energy balance and even lead to a loss of body fat mass when no snacks were consumed in-between meals (Table 1). The effect of orange juice consumption on risk of overweight and obesity is controversial with some studies showing a higher risk^{2, 33–35}, whereas others reported no effect^{36–38} or even found a lower body weight in regular fruit juice consumers compared to people with no fruit juice intake^{39, 40}. Associations between beverage intake and weight status observed in epidemiological studies are, however, not necessarily causal. Lifestyle behavior may differ between different types of beverage consumers and therefore act as an important confounder. Accordingly, 100% orange juice consumption was found to be associated with better diet quality, improved nutrient adequacy, and improved biomarkers of health in adults⁴¹. In contrast, intake of SSB was associated with a less healthy diet⁴² and high soft drink consumers also engaged in less physical activity than low soft drink consumers^{2, 43}.

Similar to the above mentioned controversial findings of cross-sectional and observational data, evidence from intervention studies on orange juice and weight gain is inconclusive. A previous study has shown that consumption of 250 mL orange juice per day for a period of 12 weeks did not adversely affect body weight or insulin sensitivity⁴⁴. This was however also true for the control group who consumed an energy and sugars-matched orange-flavored drink. In another dietary intervention trial, body weight and leptin levels decreased in overweight and obese subjects after consumption of 2×250 mL orange juice per day for a period of 12 weeks⁴⁵.

However, participants received nutritional advice to aid them in balancing out the additional calories that they were ingesting from orange juice intake. Unfortunately, it was not reported whether the subjects drank the orange juice with or in-between meals. One important difference between SSB and fruit juices is that juice is usually not consumed as a thirst quencher. Timing of consumption, i.e., with or in-between meals may therefore also be different (e.g., drinking orange juice as part of a breakfast). Unlike numerous other studies, one observational study in Canadian children specifically asked for SSB consumption in-between meals and found that this intake pattern more than doubles the odds of being overweight³⁴.

Contrary to previous interventions^{44, 45}, we used a very high amount of orange juice (about 1.28 L/d) in order to achieve a measureable impact on energy balance within the short time frame. Compared with SSB, orange juice is usually consumed in smaller amounts of only 7.8 L per capita per year (equivalent to 21 mL per day⁴⁶), in Germany. This may partly explain the lack of association between orange juice consumption and body weight in some studies. We have chosen an amount of orange juice that closely resembles the habitual intake of soft drinks in young German adults. The highest amounts are consumed by young 18–29 year old men with about 900 mL of SSB and fruit juices per day⁴⁷. This equals a habitual intake of sugar of about 96 g/d from SSB and fruit juice, being comparable to the consumed amount of sugar from orange juice in the present study. During BM-intervention, participants in the present study consumed a total of 7697 ± 1349 kcal from orange juice. Without compensation during the following meals, this would lead to a positive energy balance and equals a theoretical gain in

FM of 855 ± 150 g. Noteworthy; participants of the present study were not instructed to adapt their meals to compensate for the additionally consumed orange juice. The actual gain in FM of 1.02 ± 1.81 kg during BM-intervention confirmed this lack of caloric compensation during subsequent meals when orange juice is consumed as a snack in-between meals.

In-between meals, insulin levels decrease, and activate lipolysis that may also be important for metabolic regulation¹⁵. This is supported by protocols comparing low and high meal frequencies at the same energy intake. A lower meal frequency was associated with lower liver fat during hypocaloric⁴⁸ as well as hypercaloric diets⁴⁹. In line with metabolic improvement and a suspected loss in liver fat, serum GGT decreased with loss in FM after WM-intervention (Table 1). GGT is associated with several cardiovascular risk factors and shows a dose-response relationship with incident diabetes, even within its normal range⁵⁰.

Contrary to the finding of Meng et al. 2017, in the present study the incremental glucose area under the curve did not differ and MAGE-Index was higher with WM-intervention compared to BM-intervention. Hence we may deduce that postprandial glycemia is not attenuated by a concurrent meal when sugar is consumed in a liquid form that quickly passes the stomach into the intestine¹⁹. This is in line with no significant differences in fructosamine levels between the interventions (Table 1).

The impact of regular orange juice intake on the risk for type 2 diabetes is as controversial as its effect on the risk of obesity (for meta-analysis see⁵¹). Despite the high sugar content of orange juice, flavanones and vitamin C may even reduce cardiovascular⁴⁵ and diabetes risk⁵², possibly due to their antioxidant and anti-inflammatory activities. These components might also compensate for increased oxidative stress associated with increased glucose variability⁵³ that was shown in the present study by a higher MAGE index with WM-intervention (Table 2). Finally, the subjects of our study were young, healthy and mostly normal weight. The results can therefore not be transferred to overweight, obese, and elderly people or patients with type 2 diabetes, because impaired glucose metabolism might lead to a more pronounced impairment of postprandial insulin sensitivity with BM-intervention.

The crossover intervention is an advantage of the present study. An additional strength is the measurement of body composition with elaborate repeated measurement of ADP in order to increase MDC of fat mass. There are however some limitations that need to be addressed. Although all study participants followed an ad libitum diet, meal frequency was fixed. We therefore could not examine the voluntary intake of snacks in-between meals. Since orange juice adds to the calories consumed at meal-time⁵⁴, the loss in FM after WM-intervention is not due

to a lower energy intake with meals but likely explained by the fact that participants were not allowed to eat in-between main meals. Finally, the results of our study only refer to liquid calories and cannot be transferred to solid snacks eaten in-between meals. Although most of our participants were normal weight, our findings may be of special importance for prevention of weight gain in overweight people since the propensity of weight gain may be more pronounced in this group.

In conclusion, in order to take advantage of the health benefits of fruit juice, dietary counselling should advice to drink orange juice together with only 3 main meals rather than as an in-between meal snack. Further studies are required to verify these results and to investigate their transferability to timing of SSB intake. Moreover, studies in vulnerable groups like overweight and elderly individuals are needed to investigate the impact of SSB consumption with or in-between meals on outcome parameters like liver fat and insulin resistance.

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Authors contributions

A.B-W. designed research; F.A.H., F.B., A.N., L.G. conducted research; F.A.H. and F.B. analyzed data; F.A.H. and A.B-W. wrote the paper and had primary responsibility for final content; A.B-W., F.A.H., F.B., J.A., R.S., and R.C. discussed the data. All authors read and approved the final manuscript.

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Conflict of interest

The study was in part financed by a grant from the Baumann-Gonser-Stiftung, Bonn, Germany. R.C. received a donation from the Baumann-Gonser-Stiftung, Bonn, Germany. The remaining authors declare that they have no conflict of interest.

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Chapter III:

**Appetite control is improved by acute increases in energy
turnover at different levels of energy balance**

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Appetite Control Is Improved by Acute Increases in Energy Turnover at Different Levels of Energy Balance

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Background: Weight control is hypothesized to be improved when physical activity and energy intake are both high [high energy turnover (ET)].

Objective: The impact of three levels of ET on short-term appetite control is therefore investigated at fixed levels of energy balance.

Design: In a randomized crossover trial, 16 healthy adults (25.1 ± 3.9 y of age; body mass index, 24.0 ± 3.2 kg/m²) spent three daylong protocols for four times in a metabolic chamber. Four conditions of energy balance (*ad libitum* energy intake, zero energy balance, -25% caloric restriction, and $+25\%$ overfeeding) were each performed at three levels of ET (PAL 1.3 low, 1.6 medium, and 1.8 high ET; by walking on a treadmill). Levels of appetite hormones ghrelin, GLP-1, and insulin (total area under the curve) were measured during 14 hours. Subjective appetite ratings were assessed by visual analog scales.

Results: Compared with high ET, low ET led to decreased GLP-1 (at all energy balance conditions: $P < 0.001$) and increased ghrelin concentrations (caloric restriction and overfeeding: $P < 0.001$), which was consistent with higher feelings of hunger (zero energy balance: $P < 0.001$) and desire to eat (all energy balance conditions: $P < 0.05$) and a positive energy balance during *ad libitum* intake ($+17.5\%$; $P < 0.001$).

Conclusion: Appetite is regulated more effectively at a high level of ET, whereas overeating and consequently weight gain are likely to occur at low levels of ET. In contrast to the prevailing concept of body weight control, the positive impact of physical activity is independent from burning up more calories and is explained by improved appetite sensations. (*J Clin Endocrinol Metab* 104: 4481–4491, 2019)

Energy turnover (ET) is defined as the level of energy balance (EB), that is, a high ET is achieved at a high level of energy expenditure (very physically active) and a corresponding high energy intake (EI). The same EB can be achieved with a low ET at a low level of energy expenditure and corresponding low EI (see Fig. 1).

From a teleological point of view, energy expenditure should directly impact the control of appetite and EI (1). A

historical study led to the hypothesis that appetite is homeostatically controlled when the physical activity level is high but this control is lost at lower energy expenditure (2). This paradigm of an asymmetric control of EB with a “regulated zone” at high energy expenditure and an “unregulated zone” at low energy expenditure (3) is incompletely verified because it requires a fixed EB at different levels of energy expenditure, a condition that can be

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Abbreviations: CR, caloric restriction; EB, energy balance; EI, energy intake; Erq, energy requirement; ET, energy turnover; FMI, fat mass index; OF, overfeeding; PAL, physical activity level; REE, resting energy expenditure; SEE, sleeping energy expenditure; TEE, total energy expenditure during 24 hours; VAS, visual analog scale.

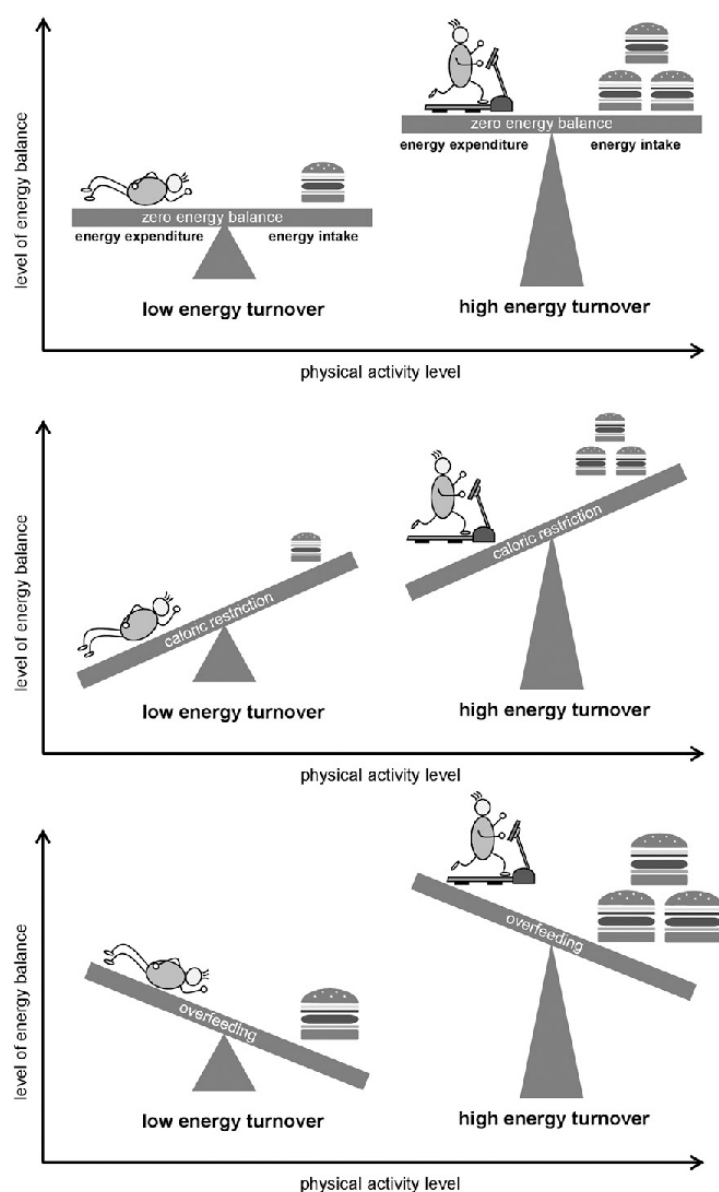


Figure 1. Graphic depiction of the ET concept.

obtained in the complex setting of a metabolic chamber. Studies under free-living conditions provide only indirect evidence for this paradigm because EB is not fixed.

Data from Stubbs *et al.* (4) suggest that there is no compensatory increase in *ad libitum* food intake in response to 9 days of medium or high exercise level leading to a negative EB compared with inactive control under free-living conditions. Alternatively, inactivity has been shown to promote excess EI and weight gain (5), sedentary time (physical inactivity) is positively associated with adipose

tissue mass (6), and exercise is known to facilitate weight control. Time spent in moderate-to-vigorous physical activity was inversely associated with prospective weight gain in young adults (7) and weight regain after weight loss (8). Additionally, a high level of structured aerobic exercise has been shown to reduce body fat mass by increasing energy expenditure, which is not compensated by an increase in EI (9).

Hunger and satiety are responses to neural and hormonal information reporting the current state of EB to key brain regions via signals such as appetite hormones (10). A dramatic reduction in energy expenditure during 1 day of sitting was not accompanied by reduced appetite signals (11). Changes in appetite-regulating hormones following acute exercise might be intensity-dependent, with increasing intensity leading to a greater suppression of orexigenic signals and greater stimulation of anorexigenic signals (12). Hunger and appetite regulatory hormones were found to be insensitive to low-intensity bouts of physical activity, suggesting beneficial implications on body weight control (13). The effect of ET modulated by different durations of low-intensity physical activity on appetite control remains unclear.

The aim of the current study was to investigate the effect of different levels of ET obtained by varying amounts of low-intensity physical activity (brisk walking) on short-term appetite control during different degrees of EB under highly controlled conditions in a metabolic chamber. We hypothesize that appetite is better controlled at high ET independent of EB.

Subjects and Methods

Study population

Sixteen healthy adults (3 women, 13 men) aged between 20 and 40 years were recruited from December 2016 to January 2018 by notice board postings at the Universities of Hohenheim and Stuttgart (both in Stuttgart, Germany) and from social networks. Exclusion criteria were chronic diseases, regular use of medication

or supplements, alternative eating habits, food allergies or intolerances, claustrophobia, and smoking. The study protocol was approved by the Ethics Committee of the State Medical Council of Baden-Württemberg, Germany (F-2016-099), in accordance with the Declaration of Helsinki. This trial was registered at ClinicalTrials.gov as no. NCT03361566. All subjects provided written informed consent before participation.

Study protocol

The randomized crossover trial comprised a highly controlled nutritional intervention for 4 weeks in every subject for which 3 days per week were spent in a metabolic chamber at the Institute of Nutritional Medicine, University of Hohenheim in Stuttgart, Germany. An outline of the study protocol is given in Fig. 2. Three different levels of daylong ET [low, physical activity level (PAL) 1.3; medium, PAL 1.6; high, PAL 1.8] were obtained by controlled walking on a treadmill in the metabolic chamber and correspondingly matched EI. Energy requirement at different levels of physical activity was determined during *ad libitum* EI. In the following, EB on intervention days was changed between zero EB, 25% caloric restriction (CR), and 25% overfeeding (OF). The order of ET levels within each EB condition was randomized as well as the order of CR and OF as the second and fourth conditions. Block randomization was conducted using computer-generated random numbers. Each EB condition started with a 3-day run-in period with controlled diet and fixed macronutrient composition to adapt macronutrient oxidation to macronutrient intake (14) and to ensure equal baseline conditions. Subjects entered the

metabolic chamber the evening before each 24-hour intervention to adapt to the environment. Daily routine was strictly controlled with wakeup at 6:00 AM, breakfast at 7:00 AM, lunch at 1:00 PM, dinner at 7:00 PM, and bedtime at 10:30 PM. The three ET days were separated by one washout day each and participants were allowed to leave the institute for 12 hours on these days.

Diet composition

Macronutrient composition was 50% carbohydrates, 35% fat, and 15% protein for all days and for each meal using a Western style diet. During *ad libitum* EI, subjects were asked to complete all meals within 30 minutes and eat until they felt comfortably full. Leftovers were weighed and the EI was calculated. Individual energy requirement (Erq) for the three levels of physical activity was measured in a metabolic chamber under *ad libitum* EI condition. On the following intervention days, participants were fed 100% Erq at EB, 75% Erq at CR, and 125% Erq at OF. Leftovers were not allowed during these periods, and all meals were consumed within 30 minutes. During the different EB conditions, the diet consisted of the same food items on chamber days, washout days, and during run-in periods, respectively. Every food item was weighted to the nearest 0.1 g for each participant according to his or her individual energy requirement using a digital scale. Individual diet composition and actual energy and macronutrient intake were calculated using Prodi® 6 software (Wissenschaftliche Verlagsgesellschaft, Stuttgart, Germany). Food was provided by the Institute for Nutritional Medicine, University of Hohenheim, and subjects were

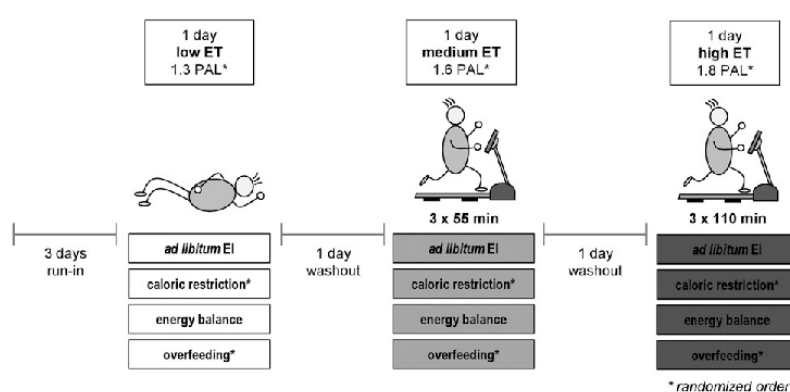


Figure 2. Study protocol of the randomized crossover intervention at three different levels of ET under four different EB conditions. Different levels of ET were achieved by walking on a treadmill at the speed of 4 km/h for either 0 min (low ET), three times for 55 min (medium ET), or three times for 110 min (high ET). Energy balance conditions were *ad libitum* EI, zero EB, caloric restriction (−25%), and overfeeding (+25%). Levels of ET during each EB condition were randomized as well as order of caloric restriction and overfeeding. Intervention days were preceded by 3 d of run-in at every EB condition and separated by one washout day each. During the entire study protocol subjects received a controlled diet with constant macronutrient relation (50% carbohydrates, 35% fat, 15% protein).

Table 1. EB Parameters for Interventions Differing in ET and EB

	<i>Ad Libitum</i> EI			CR		
	Low ET	Med ET	High ET	Low ET	Med ET	High ET
TEE, kcal/d	2480 ± 99	2873 ± 134	3320 ± 277	2258 ± 107	2653 ± 117	3098 ± 189
Steps/d	399 ± 967	17,599 ± 299	34,603 ± 1257	543 ± 894	17,609 ± 483	34,536 ± 1884
PAL	1.31 ± 0.04	1.55 ± 0.06	1.76 ± 0.09	1.27 ± 0.05	1.53 ± 0.08	1.75 ± 0.08
EI, kcal/d	2789 ± 182	3060 ± 254	3308 ± 405	1794 ± 182	2158 ± 254	2486 ± 405
EB, %	17.5	7.0	−0.3	−20.5	−19.0	−21.0

(Continued)

instructed to only consume the allocated foods, water, and unsweetened fruit or herbal tea and to abstain from any exercise during the whole study period.

24-Hour EI

The metabolic chambers are two 9 m² rooms and have a total volume of 21,000 L each (D&S Consulting Services, New York, NY). Equipment and methodology are described in detail elsewhere (15). Room temperature and flow rate were set to 24.5°C and 120 L/min, respectively. Response time correction of the metabolic chamber data were performed using z transformation (16). Total energy expenditure during 24 hours (TEE) was continuously measured (from 6:00 AM to 6:00 AM) by rates of oxygen consumption and carbon dioxide production using the Promethion integrated whole-room indirect calorimeter system (Sable Systems International, Las Vegas, NV) and by using the Weir equation (17). Twenty-four-hour urea excretion was assessed to calculate nitrogen excretion on intervention days at EB, CR, and OF to correct TEE for protein oxidation [for details, see (15)]. Owing to technical problems during data collection, full TEE data for the whole intervention period are available for 11 participants only, whereas TEE data for *ad libitum* EI are available for all 16 participants.

Appetite control

Objective and subjective appetite control were assessed. For the objective assessment concentrations of appetite-regulating hormones ghrelin, GLP-1, and insulin were measured in blood samples on intervention days during EB, CR, and OF. Using EI and TEE data, individual EBs for different ET levels were calculated as $\Delta EB (\%) = (EI / TEE \times 100) - 100$.

For subjective assessment of appetite, participants reported their sensations of hunger, fullness, and desire to eat using visual analog scales (VASs) every 2 hours during the day and at 0, 30, 60, and 120 minutes postprandial on intervention days during EB, CR, and OF. A subsample of eight subjects additionally completed

VASs on desire for something sweet, salty, and fatty at the same time points. VASs consisted of a 100-mm horizontal line with “not at all” anchored at 0 mm and “extremely” at 100 mm (18). Higher ratings indicate greater experienced sensations. Total area under the curve was calculated for appetite hormones for 14 hours (7:00 AM to 9:00 PM) and for VASs for 15 hours (7:00 AM to 10:00 PM) using the trapezoidal rule (19). Because energy requirement for CR and OF was calculated as −2.5% and +2.5% of the EI at EB, absolute values for EI differed between ET conditions (*e.g.*, −600 kcal at CR low ET and −832 kcal at CR high ET). Results on appetite hormones and VASs were therefore adjusted for differences in absolute intake of calories.

Blood sampling and analytical methods

Blood samples were taken every 2 hours during the day from 7:00 AM to 9:00 PM at EB, CR, and OF and 0, 30, 60, and 120 minutes postprandially. Plasma samples for the measurement of appetite hormones were collected in BDTM P800 tubes (Becton Dickinson, Franklin Lakes, NJ) containing a mixture of protease, esterase, and DPP-IV inhibitors to prevent hormonal degradation. Samples were stored on ice after collection and then centrifuged immediately. Total ghrelin, total GLP-1, and insulin were measured using a Bio-Plex ProTM human diabetes 3-plex kit (Bio-Rad Laboratories, Hercules, CA) containing antibody-conjugated microspheres. Analysis was performed on a Bio-Plex[®] 200 suspension array reader (Bio-Rad Laboratories) according to the manufacturers' instructions. Standard curve optimization and data analysis were performed with Bio-Plex ManagerTM software 6.1. Twenty-four hour urea excretion was determined using photometry (Beckman Coulter, Brea, CA; AU5800). Aliquots of all samples were stored at −80°C until analysis.

Body composition

Height was measured using a stadiometer (Seca 274; Seca, Hamburg, Germany) and body composition was assessed using air displacement plethysmography by the

Table 1. EB Parameters for Interventions Differing in ET and EB (Continued)

Zero EB			OF		
Low ET	Med ET	High ET	Low ET	Med ET	High ET
2296 ± 99	2847 ± 37	3210 ± 157	2340 ± 86	2846 ± 91	3271 ± 172
429 ± 910	17,703 ± 327	34,747 ± 1110	443 ± 845	17,788 ± 370	34,765 ± 1593
1.30 ± 0.03	1.57 ± 0.04	1.76 ± 0.07	1.34 ± 0.05	1.55 ± 0.05	1.74 ± 0.09
2390 ± 182	2874 ± 254	3325 ± 405	2978 ± 182	3586 ± 254	4,161 ± 405
3.4	0.0	1.6	27.6	25.4	24.2

Values are means ± SD, n = 16; steps/d, n = 13–15; PAL, n = 11; TEE and EB for zero EB, CR, and OF, n = 11. Parameters differed between interventions according to study protocol.

Abbreviation: Med, medium.

BodPod™ Body Composition System (Cosmed, Rome, Italy) at baseline. Fat mass index (FMI) was calculated as fat mass divided by height squared (kg/m^2). Weight was assessed by an electronic scale coupled to the BodPod™ system. Body weight was also measured at the beginning and end of each EB condition using a calibrated electronic scale (Seca mBCA 515; Seca). All measurements were conducted in the fasting state and in underwear.

Physical activity

Prior to the study start, which walking time and speed were needed to reach the predetermined PALs was tested. Medium and high ET were achieved through different treadmill protocols. On medium ET days, participants walked three times for 55 minutes (11 km) on a treadmill (Kettler Track 9 with software World Tours 2.0; Kettler, Ense-Parsit, Germany) at a speed of 4 km/h starting 10 minutes after meal termination. On high ET days, time on the treadmill was doubled to three times for 110 minutes (22 km) at the same speed. Besides the prescribed time on the treadmill participants remained sedentary (sitting and lying) but awake until bedtime. On the low ET days participants remained sedentary all of the time. PAL was calculated as $\text{PAL} = \text{TEE}/\text{resting energy expenditure (REE)}$ with $\text{REE} = \text{sleeping energy expenditure (SEE)} + 0.5 \times \text{SEE}$ (20) using TEE and SEE data from metabolic chambers. SEE was measured as reported by Schrauwen *et al.* (21) as the lowest energy expenditure value of three consecutive hours during sleep between 12:00 AM and 6:00 AM. Physical activity was continuously measured via step count using the triaxial activity monitor ActivPAL™ 3C, (Paltechnologies, Glasgow, UK). The ActivPAL™ uses dynamic triaxial acceleration and inclination logging technology and is a valid and reliable device (22). The ActivPAL™ was fixed in the middle of the upper thigh with waterproof patches and was worn permanently during the whole study period, and subjects were requested to refrain from exercise during this time. Data were analyzed with ActivPAL™ Professional v7.2.32

software. Owing to technical failure, one subject had no valid data of physical activity for the whole intervention and two participants had missing data for one EB condition each. For washout days ActivPAL™ data are available for n = 7 to 12 participants only.

Statistical analyses

Primary outcome variables for the present analysis are ghrelin, GLP-1, and insulin concentrations. Secondary outcomes are subjective appetite sensations (VAS) and EB during *ad libitum* EI. Calculation of the required sample size was conducted using G*Power 3.1.9.2 software (written by F. Faul, University of Kiel, Kiel, Germany) for ghrelin concentration as the primary outcome variable. King *et al.* (23) observed 29.9% lower ghrelin concentrations after exercise compared with inactivity at the same EB in a randomized crossover intervention. Based on these results, 13 participants are required to detect this difference in ghrelin concentration between low ET and high ET at the same EB (assuming a two-sided power of 95% and an α level of 5%). The statistical software R (2018) was used to analyze the data. Data evaluation started with the definition of an appropriate statistical mixed model (24, 25). The data were assumed to be normally distributed and to be heteroscedastic with respect to the different levels of EB and ET. These assumptions were based on a graphical residual analysis. The statistical model included EB (*ad libitum* EI, EB, CR, OF) and ET (low, medium, high), as well as their interaction term as fixed factors. The subject identity was regarded as a random factor. Additionally, the correlations of the measurement values due to the several intervention days were taken into account (autocorrelation). Based on this model, a pseudo R^2 was calculated (26) and an ANOVA was conducted, followed by multiple contrast tests [*e.g.*, see (27, 28)] to compare the three levels of ET within each EB condition. Deviations of ΔEB (%) during *ad libitum* EI from an equal EB were tested vs the fixed value (0%) by multiple

contrast tests also. Taking this model into account, Pearson correlations were calculated for all levels of ET combined comparing daylong insulin and ghrelin. Data are presented as mean \pm SD, and a two-sided P of <0.05 was considered to be statistically significant.

Results

Basal characteristics of the study population

Sixteen adults (3 women, 13 men) aged 20 to 32 years (25.1 ± 3.9 years) with a body mass index between 19.6 and 31.2 kg/m^2 ($24.0 \pm 3.2 \text{ kg/m}^2$) and an FMI between 1.8 and 14.1 kg/m^2 ($5.3 \pm 3.2 \text{ kg/m}^2$) participated in the trial. According to World Health Organization criteria, one woman and four men were overweight and one woman was obese. Taking body composition into account, two women and two men had an FMI above the age- and sex-adjusted 95th percentile (29). Body weight did not change during any of the four EB conditions (all $P > 0.05$, data not shown).

Parameters of EB

Components of EB are shown in Table 1. Compared with low ET, TEE was 24% higher with medium ET and 40% higher with high ET during EB. EI and TEE differed between all ET levels (all $P < 0.01$). PAL and steps per day were similar between same ET levels (all $P > 0.05$) except for a lower PAL at low ET during CR compared with OF ($P < 0.01$). As intended by study protocol, EB was negative during CR, positive during OF (all $P < 0.001$), and equal during EB. Steps per day were similar on all washout days following the interventions (all $P > 0.05$, data not shown).

Appetite hormones

Results for daylong concentrations of appetite hormones are shown in Fig. 3. Ghrelin concentrations were higher with lower ET during CR and OF [all $P < 0.05$, Fig. 3(a)] except for a nonsignificantly lower ghrelin with high ET compared with medium ET during CR. GLP-1 concentrations were lower, with lower ET during all EB conditions [all $P < 0.01$, Fig. 3(b)] except for nonsignificant differences between low and medium ET during CR and between medium and high ET during OF. Insulin concentrations were higher with lower ET during OF (low vs high ET and medium vs high ET, both $P < 0.01$) and EB (medium vs high ET, $P < 0.05$), whereas no differences were found between insulin concentrations at three levels of ET during CR despite a tendency of higher concentrations with low ET compared with high ET [$P = 0.058$; Fig. 3(c)]. Daylong insulin and ghrelin concentrations were negatively correlated, with $r = -0.34$ ($P < 0.001$).

Subjective appetite ratings

Results for subjective appetite ratings are shown in Table 2. Hunger was higher with lower ET during EB (all $P < 0.01$), whereas no differences in hunger were found between levels of ET during CR and OF. Fullness was lower with lower ET during all EB conditions (all $P < 0.05$) except for nonsignificant differences between medium and high ET during EB and OF. Desire to eat was higher with lower ET during all EB conditions (all $P < 0.05$) except for nonsignificant differences between low and medium ET for CR and OF and between medium and high ET for EB and OF. Desire for something sweet, salty, or fatty was assessed in a subsample of $n = 8$ but showed no consistent results (data not shown).

EB during *ad libitum* EI

In line with the findings for appetite hormones and subjective appetite ratings, *ad libitum* EI led to a more positive EB at a lower ET (low vs medium and low vs high ET, both $P < 0.01$, Fig. 4). During low and medium ET, EB was positive (+17.5%, $P < 0.001$ and +7.0%, $P < 0.05$, respectively), but with high ET, EB was equal ($P > 0.05$).

Discussion

The current study confirms the hypothesis that appetite control is improved at higher levels of ET achieved by low-intensity physical activity. The results are independent of EB and are verified by changes in endocrine signals, subjective appetite ratings, and *ad libitum* EI: ghrelin concentrations, hunger, and desire to eat were higher, GLP-1 concentrations were lower, and EB during *ad libitum* EI was positive with low ET compared with high ET.

In a prospective study by Hume *et al.* (30), gain in fat mass within a 2- to 3-year period was predicted by low ET but not by energy surfeit at baseline using state-of-the-art methods for assessment of total energy expenditure. However, the study was criticized for not measuring real ET but simply examining baseline energy expenditure of weight-stable persons against long-term changes in body fat (31). Nevertheless, the results by Hume *et al.* are in line with the present randomized intervention study that carefully measured ET at different levels of EB in a whole-room calorimeter and that has demonstrated improved appetite control with increased ET.

The present findings indicate a relationship between decreasing ghrelin levels in the observed concentration range and a reduced food consumption. These findings are confirmed by a meal test trial where physiological ghrelin levels were closely correlated with hunger during the postprandial period and predicted the amount of

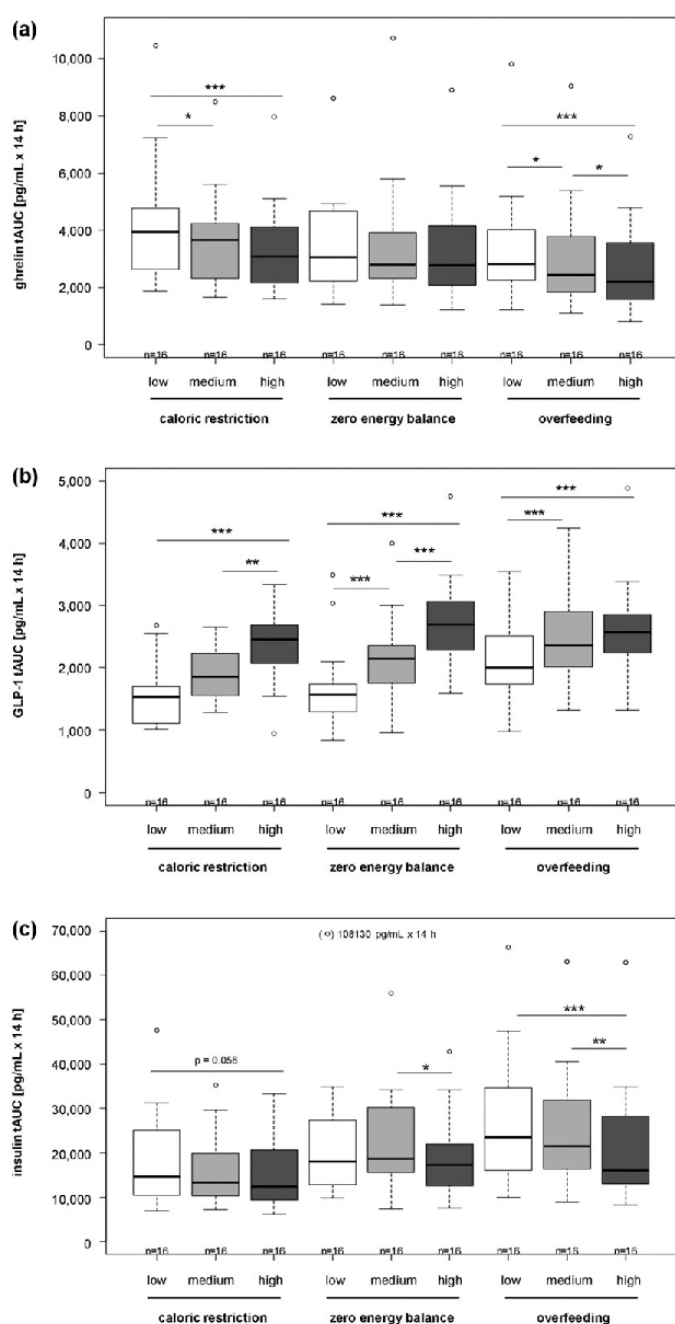


Figure 3. Comparison of daylong appetite hormone responses for ghrelin (a), GLP-1 (b), and insulin (c) as tAUC for 14 h between interventions with differing ET and differing EB. Mean \pm SD; n = 16. * P < 0.05, ** P < 0.01, *** P < 0.001, by linear mixed model with multiple contrast tests. tAUC, total area under the curve.

subsequent food consumption (32). Ghrelin concentrations are known to rise in the fasted state until meal initiation and to fall afterward proportional to the ingested energy content (33). Reduced ghrelin concentrations with

increasing ET could therefore be due to higher energy content of meals with increasing ET. It was shown that stomach distention does not directly affect declining ghrelin concentrations (34), indicating that the size or volume *per se* has no effect on postprandial ghrelin suppression. Because insulin is essential for postprandial ghrelin suppression (35), the inverse correlation between insulin and ghrelin concentrations in the present trial supports an indirect anorexigenic effect of insulin via ghrelin suppression. Because daylong insulin secretion decreased with higher ET [Fig. 3(c)], the direct anorexigenic effect of insulin may be of minor importance. The anorexigenic effect implied by high GLP-1 [Fig. 3(b)] and the suppressed orexigenic effect of ghrelin [Fig. 3(a)] with a higher ET both seem to be sufficient to promote satiety and decrease desire to eat (Table 2). These effects were also found with CR and OF [Fig. 3(a) and 3(b)], and therefore the impact of ET on appetite control seems to be independent of EB.

Beyond ET, the type of energy deficit might also impact appetite control. Equal energy deficits (-1100 kcal/d) induced by 90 minutes of exercise at 70% maximal oxygen uptake or CR differently affected appetite control and food intake in healthy males, with only CR leading to elevated ghrelin concentrations and a compensation of the energy deficit (23). A similar effect in women was already reported earlier (36). The current study confirms these findings despite a lower intensity of physical activity because a high ET achieved by brisk walking led to lower appetite and *ad libitum* EI compared with low ET. Similarly, no compensatory increases in acylated ghrelin, appetite, or EI were observed after an acute brisk walking session (37). This corresponds to the idea of “exercise-induced anorexia” after an acute bout of moderate-to-vigorous exercise, which is characterized by a short-lived reduction in hunger and a delayed onset of EI but without a full compensation of the energy deficit

Table 2. Comparison of Daylong Subjective Appetite Feelings From Visual Analog Scales (All tAUCs) Between Interventions With Differing ET and Differing EB

	CR			Zero EB			OF		
	Low ET	Medium ET	High ET	Low ET	Medium ET	High ET	Low ET	Medium ET	High ET
Hunger, mm × 15 h	660 ± 146	578 ± 139	542 ± 201	559 ± 74	466 ± 71 ^a	409 ± 46 ^{a,b}	412 ± 347	348 ± 30	303 ± 92
Fullness, mm × 15 h	470 ± 79	537 ± 82 ^c	630 ± 85 ^{b,b}	535 ± 72	636 ± 114 ^d	710 ± 178 ^a	709 ± 120	813 ± 123 ^c	879 ± 145 ^a
Desire to eat, mm × 15 h	678 ± 151	630 ± 200	502 ± 77 ^{a,e}	574 ± 112	437 ± 71 ^a	391 ± 68 ^a	381 ± 148	330 ± 82	277 ± 107 ^c

Values are mean ± SD, n = 16; linear mixed model with multiple contrast tests.

Abbreviations: Med, medium; tAUC, total area under the curve.

^aP < 0.001, significantly different from low ET.

^bP < 0.01, significantly different from medium ET.

^cP < 0.05, significantly different from low ET.

^dP < 0.01, significantly different from low ET.

^eP < 0.05, significantly different from medium ET.

(38). It is discussed that “exercise-induced anorexia” is mediated by lowered concentrations of acylated ghrelin and elevated concentrations of GLP-1 and PYY [for review, see (39)]. The results of the present trial confirm these findings, as ghrelin was reduced and GLP-1 increased with high ET.

At a low ET, the macronutrient intake may have been too small and therefore not sufficient to stimulate GLP-1 secretion and ghrelin suppression. A threshold effect could therefore explain the lack in satiety (Table 2) and energy overconsumption during *ad libitum* EI (Fig. 4). This assumption is supported by the finding of higher prospective weight gain with snacking behavior (frequent consumption of small amounts of food) under free-living conditions (40).

In line with our findings, Stubbs *et al.* (41) showed in a metabolic chamber trial that *ad libitum* EI does not adequately adapt to a reduction in physical activity (PAL 1.8 to 1.4) and thus led to a positive EB. In the current study, during *ad libitum* EI, EB was 17.5% positive with low ET. Assuming 13.1 kcal/g for energy content and energy cost of fat mass synthesis (42), an exclusive gain in fat mass would resemble about +32 g for 1 day of inactivity. Therefore, an inactive lifestyle facilitates caloric overconsumption and thus weight gain.

Low-intensity physical activity was chosen to increase the PAL, simulating daily activities without the effects of vigorous exercise on metabolism and avoiding fatigue or pain in persons with very low fitness. Furthermore, low-intensity physical activity is considered to play an important role in promoting health and may be implemented more easily in public health strategies than high-intensity exercise (43). In the current study, a PAL of 1.76 was required to achieve the “regulated zone” of energy expenditure, where appetite is matched to actual energy demand. The setting of a 9-m² metabolic chamber only allows physical activity on a treadmill and not manifold physical activities (like housekeeping or taking the stairs) as compared with free-living conditions. Therefore, the amount of steps that is necessary to achieve a high ET was $\approx 34,500$ steps per day. Under free-living conditions, the appropriate amount of steps per day would therefore be much lower. In line with our findings, the World Health Organization in 2001 recommended a PAL target of ≈ 1.75 that represents an individual whose occupation involves regular physical activity (*i.e.*, regular exercise) whereas a PAL of 1.4 represents a sedentary individual (44). Because the difference between a PAL of 1.4 and 1.75 equates to an additional energy expenditure of 490 kcal/d (with an REE of 1400 kcal/d), a public health recommendation of only 150 of 300 min/wk moderate intensity physical activity from 2010 (45) is unrealistically low (43, 46, 47). As an outcome of the International Association

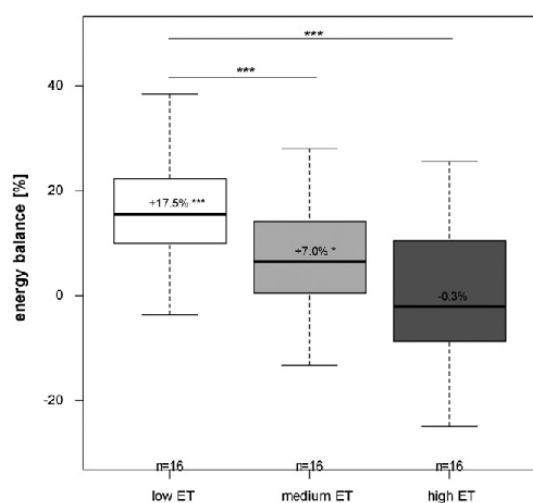


Figure 4. Comparison of EB (%) between interventions with differing ET during *ad libitum* EI. Mean \pm SD; n = 16. * $P < 0.05$, *** $P < 0.001$, by linear mixed model with multiple contrast tests.

for the Study of Obesity First Stock Conference, a consensus statement was published that confirmed the necessary PAL of 1.7 or 45 to 60 min/d moderate intensity physical activity to prevent unhealthy weight gain (46). Accordingly, another study demonstrated that a habitual PAL of ≥ 1.62 was associated with improved appetite control compared with a PAL of ≤ 1.49 (48). Because in our study this high level of physical activity was obtained at a low intensity (walking at 4 km/h), this led to an unrealistic long duration of activity. Future studies need to investigate whether a higher intensity of physical activity at a lower duration is equally sufficient for optimal control of EB. Importantly, note that our study was conducted in mostly lean young subjects and a typical Western diet was used (energy density 5.8 kcal/g). Therefore, the PAL necessary to obtain EB may be different in subjects with obesity and a less energy-dense diet.

The role that exercise can play in energy expenditure is commonly exaggerated (49). Our findings show that the positive impact of physical activity on weight control is partially independent of burning up more calories and is additionally explained by an improvement in appetite sensations. Furthermore, increasing physical activity may be easier for most people than eating less [for review, see (50)]. Therefore, maintaining an equal EB due to achieving a high ET seems to be easier compared with obtain a low ET. However, a high level of energy expenditure and thus a high ET may also be a risk factor for future weight gain when ET cannot be maintained in the long term. Thus, a gain in FM is observed with detraining in athletes (51) or postpregnancies (52). Additionally, increasing ET by exercise in people with obesity led to a

compensatory increase in EI that alleviated the beneficial effect of exercise on body weight (53).

The extensive study protocol in randomized crossover design and the highly standardized metabolic ward conditions are a strength of our study. Nevertheless, there are also some limitations that should be addressed. We measured total ghrelin concentrations, which may mask relevant changes in acylated ghrelin that seems to be essential for appetite control (37). However, it was found that the ratio of acylated and total ghrelin is relatively robust, rendering total ghrelin an appropriate surrogate (33). Because only acute effects of changes in ET were investigated in the present trial, the results cannot be transferred to long-term habitual high or low ET. In daily life, ET is highly variable from day to day. For example, brief periods where EI far exceeds energy expenditure last from one meal to several days and regularly occur over the weekend (54). In line with our results, others have found that individuals with higher habitual PALs better adjust EI in response to EB perturbations compared with habitually inactive individuals (48, 55). However, long-term interventions are needed to confirm the present findings. Because only three women were investigated in the current study, sex differences in appetite control could not be analyzed. The impact of sex on appetite control remains unclear, with some studies showing sex differences [for review, see (39)] whereas others did not (55).

In conclusion, a high ET improves appetite control, reflected by changes in hormonal biomarkers, including increased GLP-1 and decreased ghrelin and insulin concentrations independent of EB. These results indicate an asymmetric control of appetite where reduced energy expenditure was not compensated by an appropriate adaptation in EI. In contrast to the prevailing concept of body weight control, the positive impact of physical activity is not completely explained by burning up more calories but, at certain volumes, also involves improving appetite control. Importantly, for prevention of weight gain a PAL of 1.76 does not require vigorous physical activity or exercise but can be obtained by low-intensity physical activity (*e.g.*, reached by occupational activities such as postal carriers or nursing professions).

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analyzed metabolic chamber data; F.A.H. and A.B.-W. wrote the paper and had primary responsibility for the final content; M.H. gave statistical advice and wrote R code; A.B.-W., F.A.H., F.B., M.J.M., and J.E.B. discussed the data; and all authors read and approved the final manuscript.

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Data Availability: The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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Chapter IV:

General Discussion

The present thesis examines the impact of meal frequency in a free-living intervention with consumption of orange juice together with three meals/d or in-between three meals/d on the regulation of energy intake (Chapter II). In a second study, the impact of energy turnover at different levels of energy balance on appetite control was investigated in a strictly controlled intervention under metabolic ward conditions (Chapter III).

Impact of meal frequency on energy intake

The present thesis has shown that an increased meal frequency due to the consumption of a considerable amount of orange juice in-between three meals/d leads to a gain in fat mass. In contrast, a low meal frequency due to the consumption of the same amount of orange juice together with three meals/d resulted in no change in fat mass. The observed differences seem to be explained by no compensation of energy intake at the following meal when orange juice is consumed in-between meals compared to with meals resulting in an elevated total energy intake with higher meal frequency.

In line with these findings, acute meal tests have shown that orange juice preloads did not lead to reduced energy intake in the following meal resulting in a higher total energy intake compared to a non-caloric or no preload (Almiron-Roig & Drewnowski, 2003; DellaValle *et al.*, 2005). Furthermore, it has been shown that an increased meal frequency of 6, 8 or 14 meals/d leads to enhanced hunger resulting in increased energy intake and thus a positive energy balance (Munsters & Saris, 2012; Ohkawara *et al.*, 2013; Perrigue *et al.*, 2016). In the current study (Chapter II), the consumption of orange juice in-between meals did not change body weight but resulted in an increase of fat mass (FM) after two weeks. This FM gain approximately reflected the energy content of the consumed orange juice. These findings in a long-term free-living intervention therefore verify the results of previous short-term trials on the impact of an increased meal frequency on the control of energy balance.

A possible explanation of the gain in FM only with in-between meal consumption of orange juice is the enhanced gastric distention when the juice is consumed with meals which could lead to a reduced energy intake during the meal compared to meals without orange juice consumption. In 28 lean men, it was observed in an acute meal test that a higher volume (600 mL) of a milk shake due to incorporated air led to a greater reduction in hunger and 12% lower energy intake at the following meal compared to an isoenergetic milk shake of 300 mL with the same energy density (Rolls *et al.*, 2000). The volume of a meal or beverage is detected by gastric stretch receptors signaling to the brain via the vagus nerve with greater gastric distension leading to reduced hunger and energy intake (Paintal, 1954). Moreover, the gastric emptying rate of a liquid is linearly related to its energy density with low energy dense liquids emptying faster from the stomach compared to liquids with higher energy density (Calbet & MacLean, 1997). Thus, consumption of a caloric beverage with the meal may lead to a faster and longer lasting activation of gastric stretch receptors, higher satiety and consequently reduced meal size compared to consumption of a non-caloric beverage with the same meal. In the current trial, 426 ± 74 mL of orange juice were consumed with each meal during with meal intervention compared to *ad libitum* water consumption with meals during in-between meal intervention. The effect of stomach distension due to beverages is

however short-lived as it was observed that 30 min after the consumption of 600 mL water only 44 mL can be found in the stomach (Corney *et al.*, 2016). Accordingly, consumption of orange juice in-between meals (≥ 2 h after the meals; 426 ± 75 mL/portion) was unlikely to have an effect on the following meal size since intervals between juice intake and following meal consumption were several hours. Furthermore, it was shown that small meals with an energy content of 239 – 280 kcal have low to no influence on appetite, hormonal responses or subsequent energy intake (Porrini, 1997; Marmonier *et al.*, 1999). It can therefore be assumed that very small meals are no adequate physiological load the body is able to detect and therefore appetite is not reduced in response to small meals (Leidy & Campbell, 2011). In the present study, the energy content of orange juice consumed in-between meals was only 183 ± 32 kcal per serving. Thus an effect on satiety or reduced energy intake at the following meal after several hours seems unlikely.

Another possible explanation for the gain in FM with orange juice consumed in-between meals is a prevention of a decrease in insulin levels between eating occasions. This post absorptive drop in insulin levels is important because it activates lipolysis and fat oxidation (Astrup & Raben, 1995). The finding of a gain in FM with in-between meal intervention may therefore, at least partially, be mediated by suppressed lipolysis. However, this hypothesis cannot be verified with the current data since neither insulin levels were measured continuously during the intervention days to detect a decrease in insulin secretion (cumulative 24h C-peptide excretion did not differ between interventions) nor free fatty acids or fat oxidation were assessed. Comparing meal-induced changes in ghrelin concentrations in type 1 diabetic patients and healthy subjects, it has been shown that insulin is also essential for prandial ghrelin suppression (Murdolo *et al.*, 2003). Since increasing ghrelin levels are associated with hunger and meal initiation decreasing levels are related to reduced hunger and meal termination. In a cross-over intervention by Solomon *et al.* (2008), 5 healthy males underwent three different 8h feeding regimes: fasting, low meal frequency (two meals) and high meal frequency (12 meals). Total energy intake was kept the same with low and high meal frequency and meals within trials were isoenergetic. During low meal frequency, an

inverse association between insulin and ghrelin was found but this relationship was not observed with high meal frequency. Since the inverse relationship was also seen during fasting this indicates that only minor variations in insulin are required to effect ghrelin levels. Nonetheless, during the high meal frequency the insulin-ghrelin relationship seems to be disrupted (Solomon *et al.*, 2008) which could result in enhanced energy intake. In the present study (Chapter II), neither ghrelin nor subjective hunger were assessed to investigate this effect. However, high meal frequency comprised only 6 and not 12 eating occasions/d with the 3 main meals having a larger energy content than the orange juice snacks. Thus it remains uncertain whether the insulin-ghrelin relationship may be affected by this eating pattern in the same way as observed by Solomon *et al.* (2008) and whether energy intake with in-between meal consumption was increased due to reduced insulin-induced ghrelin suppression.

The amount of orange juice used in the current study was high (20% of daily energy requirement; ~1.28 L/d) but representative for the sugary drink consumption of German males aged 18-29 years (Rabenberg & Mensink, 2013). In Germany, the highest amount of fruit juice is consumed by adolescents aged 14-18 years with 460 g/d for boys and 383 g/d for girls and by young adults aged 19-24 years with 366 g/d for men and 341 g/d for women (Max Rubner-Institut, 2008). In particular, average orange juice consumption in Germany is much lower and only about 21 mL/d (Sennewald & Heitlinger, 2015). Since in-between meal consumption of SSBs and e.g. sugar sweetened coffees is increasing, liquid snacks contribute to a considerable proportion of daily energy intake (Zizza *et al.*, 2001). The consumption of high amounts of SSBs is not only a risk factor for weight gain but also for development of type 2 diabetes (The InterAct consortium, 2013; Imamura *et al.*, 2015) and dental caries (Bernabé *et al.*, 2014). Limitation of sugar containing beverage consumption could be achieved through situational prevention programs by the government including for example the simplified labeling of high sugar containing beverages or the introduction of taxes on SSBs. In other countries it has already been shown that sugar taxes can reduce the sales and consumption of SSB as well as the sugar content of SSBs since manufacturers change their formulations to avoid taxation (Bíró, 2015; Batis *et al.*, 2016; Silver *et al.*, 2017) and that traffic light labeling of foods

supports people in making healthier food choices (for a review of both strategies see (Thavorncharoensap, 2017)). Because fruit juice also contains favorable substances as vitamin C and secondary phytochemicals like flavonoids, the German Nutrition Society (DGE) allows the replacement of one portion of fruit by a glass of fruit juice to achieve the recommended amount of 5 servings of fruits and vegetables per day. Taking the high sugar content of fruit juice into account, this replacement should indeed only occur occasionally (DGE, 2007). On the basis of the current orange juice intervention, this recommendation should be extended to include a preferential fruit juice intake together with one of only three meals per day.

Since we have only investigated liquid calories consumed in-between meals our findings cannot be transferred to solid snacks. Due to the results of several acute meal test trials investigating the effect of meal frequency it is however likely that also solid snacks eaten in-between meals hold a potential to contribute to a positive energy balance (Munsters & Saris, 2012; Ohkawara *et al.*, 2013; Perrigue *et al.*, 2016). Accordingly, all kinds of snacks in-between meals should be avoided although the potential of overconsumption with liquid calories appearing even more problematic due to the lower satiating effect compared to solid snacks (Flood-Obbagy & Rolls, 2009; Mattes & Campbell, 2009). A potential mechanism for the higher satiating value of solid foods compared to beverages may be chewing and thus eating rate. Liquid calories which can simply be gulped are typically consumed faster than solid calories leading more easily to overconsumption (de Graaf, 2011) whereas increased chewing has been demonstrated to result in decreased energy intake and ghrelin concentrations as well as enhanced GLP-1 and CCK levels (Li *et al.*, 2011). This positive relationship between eating rate and energy intake seems to be mediated by the oro-sensory exposure time which is too short with consumption of liquid calories to influence satiety (de Graaf, 2011).

All effects of the present study were observed in young and healthy adults and thus may vary in more vulnerable groups like obese or older subjects and patients with type 2 diabetes. It was shown that gastric emptying in elderly people is reduced by 26-30% compared to younger subjects contributing to reduced hunger (Clarkston *et al.*, 1997).

This could result in an attenuated impact of in-between meal consumption of sugar containing beverages in older subjects. Although no effect of high amounts of orange juice with or in-between meals on insulin sensitivity was found in the current study, the same amount of cola resulted in higher daylong glycaemia and glycemic excursions as well as reduced C-peptide excretion indicating reduced glycemic control in young and healthy adults (Büsing *et al.*, 2019). These effects may even be more pronounced in subjects with impaired glucose tolerance or diabetes. In overweight and obese people, the lack of compensation for liquid calories may also be more pronounced compared to lean subjects due to an impaired appetite control (Huda *et al.*, 2006). Therefore, studies with greater sample sizes and of longer duration are needed to investigate the impact of sugar containing beverage consumption with or in-between meals in these vulnerable groups.

Impact of energy turnover on energy intake and appetite control

This thesis has shown that appetite control is improved by acute increases in energy turnover independent from energy balance. Energy turnover was increased by low intensity physical activity but a physical activity level of 1.76 was required. Improved appetite control with a high energy turnover was verified by lower ghrelin and higher GLP-1 concentrations and correspondingly reduced hunger and desire to eat as well as an equal energy balance during *ad libitum* energy intake. The 'Mayer-Hypothesis' of an asymmetric appetite control (Mayer *et al.*, 1956) was therefore verified using strictly controlled conditions in a metabolic chamber. The present thesis thus confirms the role of physical activity in body weight regulation.

With low energy turnover at *ad libitum* energy intake a +17.5% positive energy balance was observed. Meals with low energy content of 239 – 280 kcal have little to no effect on satiety (Porrini, 1997; Marmonier *et al.*, 1999) as discussed in the previous section. However, in the present trial the required energy content per meal during low energy turnover was 791 ±110 kcal. This should have been sufficient to promote satiety. As also discussed in the previous section, meals with low volume of 300 vs. 600 mL have little

to no effect on satiety (Rolls *et al.*, 2000). In line with this finding, a study in 20 healthy men has shown that a high volume preload (450 vs. 150 mL) resulted in reduced subjective appetite irrespective of energy density (0.3 vs. 1.0 kcal/mL) (Gray *et al.*, 2002). When the meal size needed to meet the energy requirement is too small then no sufficient gastric distention and thus satiety response will occur although enough energy is ingested. Eating will then be continued until satiety is achieved by a large enough meal. This could have led to the positive energy balance with low compared to high energy turnover, because the volume of the meals was low since energy density of the diet was high (5.77 kcal/g). It is known that energy intake is also affected by energy density of the diet with a more energy dense diet leading more easily to passive overconsumption (Beaulieu *et al.*, 2018). In the present intervention (Chapter III), the high energy density of the diet decreased the needed volume of food inside the stomach to meet energy requirement which may have amplified overconsumption. Therefore, it is possible that the energy balance would be less positive with a lower energy dense diet since a higher volume of food is needed to meet energy needs. On the other hand, with a higher energy turnover the energy requirement is increased making it more difficult to eat more than is needed (Manore *et al.*, 2017). A recent study compared an ultra-processed and an unprocessed diet over 14 days in a randomized cross-over design in 20 healthy adults and found that the ultra-processed diet leads to a higher energy intake and weight gain whereas the unprocessed diet resulted in weight loss (Hall *et al.*, 2019). This finding may be due to an increased energy density of the ultra-processed food (2.15 kcal/g vs. 1.15 kcal/g) and an enhanced eating rate compared to the unprocessed diet (Hall *et al.*, 2019). In 16 lean and 14 obese Chinese subjects it has been shown that enhanced chewing from 15 to 40 chews/bite leads to reduced energy intake and ghrelin levels as well as increased GLP-1 and CCK concentrations (Li *et al.*, 2011). In 13 men and 24 women, the eating rate of 45 commonly consumed foods and subsequent energy intake were measured and eating rate was positively correlated with energy intake and inversely with energy density suggesting that rapid ingestible foods promote overconsumption (Viskaal-van Dongen *et al.*, 2011). Therefore, the weight gain with an ultra-processed diet observed by Hall *et al.* (2019) may have been caused by reduced chewing and increased eating rate. This relationship seems also plausible since

competitive eating (eating as much as possible in a short time) mostly is held with fast food i.e. ultra-processed food and not with unprocessed food items. Thus, avoiding ultra-processed food may be an effective strategy in weight management and obesity treatment (Hall *et al.*, 2019). In the study included in this thesis (Chapter III), the energy density of the diet was even higher with 5.77 kcal/g and ultra-processed food from a typical Western Diet was provided (e.g. frozen pizza, cookies, candy bars, milk shakes, fruit yoghurts, etc.). Since the energy density of the diet was the same on all intervention days neither eating rate nor chews per bite are expected to differ between energy turnover interventions and thus no bias on *ad libitum* energy intake is expected. Taken together, these findings suggest that aside from energy turnover also the kind of the consumed diet plays a role in the regulation of energy balance. When consuming a Western Diet with high content of ultra-processed foods, high energy density and low content of fiber, a higher PAL may be required for maintaining an equal energy balance compared to an unprocessed diet rich in insoluble fiber and low in energy density. Further studies are needed to investigate this association.

The current trial comprised only 24h-interventions and it remains unclear whether the effect of a high energy turnover is preserved throughout the next day. Measurements of hormones and subjective appetite as well as energy expenditure by room calorimetry were only done on the intervention days. Since the *ad libitum* energy intake on the following washout days was assessed and subjects wore the *activPAL* permanently throughout the whole study period the approximate energy balance during washout days could be calculated. No differences in daily steps on washout days were observed between energy turnover or energy balance conditions (see Chapter III). Energy balance on washout days ranged between +9.1 – +17.8% and also did not differ between energy turnover or energy balance conditions ($p>0.05$). These results suggest that the effect of energy turnover on appetite control is not compensated the day after. However, washout days were not intended to be analyzed and quality of energy expenditure data from the *activPAL* is not comparable to metabolic chamber data quality. Furthermore, subjects were not allowed to exercise on washout days and had different duties to perform (e.g. attending lectures, go to work, having a day off) which may have influenced energy

balance. Other studies observed that people with high habitual PAL better compensate energy intake of preloads compared to habitually inactive individuals suggesting a chronic improvement of appetite control with a high level of habitual physical activity (Beaulieu *et al.*, 2017; Dorling *et al.*, 2018). But long-term interventions are needed to confirm these findings.

A PAL of 1.76 was needed to achieve an equal energy balance in the present study. As discussed in Chapter III, this is consistent with the consensus of the IASO 1st Stock Conference where a PAL of 1.7 or 45-60 min moderate intensity physical activity per day is recommended for the prevention of weight gain (Saris *et al.*, 2003). This recommendation is confirmed by the findings of an observational study by Shook *et al.* (2015) examining 421 adults with differing activity levels over one year. Energy balance and thus body weight were maintained when $\geq 63 \pm 22$ min/d of moderate to vigorous physical activity were performed (Shook *et al.*, 2015). However, even the much lower level of 150 min of moderate intensity or 75 min of high intensity physical activity per week, as currently recommended by the WHO (World Health Organization, 2010), is not achieved by 57% of Germans (Froböse *et al.*, 2018). As discussed above, this low level of physical activity, especially together with a diet high in ultra-processed food, may not be enough to achieve an equal energy balance and is likely to result in weight gain. A longitudinal analysis of seven cohort studies has shown that the average weight gain in Germany is age-dependent with young men experiencing the highest rate of weight gain per year (Haftenberger *et al.*, 2016). Since young German men are consuming the highest amounts of sugar containing beverages, alcohol, sweets and meat products and the lowest amounts of fruits and vegetables (Max Rubner-Institut, 2008) the high energy density of the diet may explain the increased risk for weight gain in this group. In the present thesis it was demonstrated that the beneficial effects of physical activity are explained by improved appetite control. In order to achieve this, no vigorous exercise was needed but brisk walking was sufficient as long as a PAL of 1.76 was reached. Accordingly, small changes in daily routines and increasing daily activities as using active transportation, taking the stairs or going for a walk during lunch break can contribute to improve energy balance. The effect of those activities must not be underestimated since

the amount of more intensive exercise needed to reach a PAL of 1.76 can be reduced when daily life activities are increased. Hence, already a more active lifestyle may help to attain weight maintenance due to improved appetite control. One strategy for obesity prevention is therefore to facilitate enhanced everyday activities by situational preventions like city planning. For example, making cities more cycling-friendly (e.g. construction of attractive cycle paths or bicycle boulevards) may encourage people to increase the use of active transportation (Giles-Corti *et al.*, 2016). This could be a first step to enhance the individual energy turnover and thus improve appetite control also because increasing physical activity is suggested to be easier for most people than reducing energy intake to maintain energy balance (Hill *et al.*, 2012; Hand *et al.*, 2015).

Homeostatic appetite control is proposed to be modulated by physical activity in a dual-process action where enhanced energy expenditure increases the drive to eat but also increases the post-meal satiety resulting in a better matched energy intake compared to energy expenditure (Beaulieu *et al.*, 2018). Therefore, inactivity leads to decreased post-meal satiety enhancing the risk for overconsumption and weight gain. Also body composition seems to have an impact on this dual-process action of physical activity. In a meta-analysis it was shown that with increasing body fat insulin sensitivity of the central nervous system decreases leading to blunted satiety signaling in overweight compared to lean subjects (Flint *et al.*, 2007). When satiety signaling is already impaired due to an elevated fat mass inactivity additionally increases the risk of overconsumption (Beaulieu *et al.*, 2018). Inactive obese subjects are thus at increased risk for further weight gain and may benefit more from increasing physical activity compared to inactive lean subjects.

The result of an improved appetite control with high energy turnover raises the question of potential mechanisms by which physical activity can influence perceived hunger and subsequent energy intake. This question was already addressed in a review, that identified interleukin-6 (IL-6), among other factors, as potentially mediating the impact of exercise on appetite hormones (Hazell *et al.*, 2016). In 8 males, higher IL-6 concentrations were found after high intensities of running which were positively

correlated with GLP-1 and negatively with overall appetite (Islam *et al.*, 2017). Several human and *in vitro* trials have shown that exercise results in considerable increases in circulating IL-6 and that muscle cells *per se* secrete IL-6 (Febbraio & Pedersen, 2002). In murine models, exercise-induced high concentrations of IL-6 were shown to increase GLP-1 secretion from intestinal L-cells and pancreatic alpha-cells resulting in enhanced insulin secretion, which may also increase satiety (Ellingsgaard *et al.*, 2011). The effect of reduced appetite could be mediated directly by GLP-1 signaling via the vagus nerve to the solitary tract nucleus in the brain stem (Holst & Deacon, 2005) and the solitary tract nucleus neurons in turn signal to the arcuate nucleus and the paraventricular nucleus of the hypothalamus (Larsen *et al.*, 1997) whereby energy intake is suppressed. Alternatively, reduced appetite by exercise could be mediated by GLP-1 indirectly due to increased insulin secretion which then in turn can directly inhibit NPY/AgRP-neurons in the arcuate nucleus and reduce hunger and energy intake (Kalra *et al.*, 1999) as well as indirectly suppressing ghrelin secretion (Murdolo *et al.*, 2003). The potential mechanism by which physical activity improves appetite control may therefore be an increased GLP-1 secretion due to increased IL-6 secretion from skeletal muscle. However, it must be taken into account that the physical activity in the present study (Chapter III) was only brisk walking and that high intensity exercise which involves large muscle mass is needed for relevant IL-6 secretion (Febbraio & Pedersen, 2002). During low intensity physical activity little to no impact on muscle-induced IL-6 is expected. This suggests that an alternative mechanism linking low intensity physical activity and appetite control exists. Also other potential mechanisms (e.g. lactate production, increase in body temperature, elevated free fatty acids) raised in the review by Hazell *et al.* (2016) are unlikely to occur at low intensity physical activity. Furthermore, metabolites like IL-6 and lactate are acutely produced upon muscle activity which does not explain the observed chronic improvement in appetite control in individuals with habitual high PAL (Beaulieu *et al.*, 2017; Dorling *et al.*, 2018). A recent review by Grannell *et al.* (2019) concluded that it is unlikely that there is a muscle-specific signal which affects appetite since the association between muscle and energy intake is likely reflective of energy utilization indicating that it is overall energy expenditure that seems to determine energy intake. The authors assume that AMP-activated protein kinase (AMPK) activity could signal

energy utilization to the brain because this enzyme is responsible to restore energy homeostasis and thus shows increased activity during physical activity as well as food deprivation (Grannell *et al.*, 2019). Hypothalamic AMPK mediates incoming peripheral stimuli and influences the response of arcuate nucleus neurons thus playing an important role in central appetite control (López *et al.*, 2016). However, it remains uncertain whether distal AMPK activity due to system wide energy expenditure is coupled to central AMPK activity in the hypothalamus (Grannell *et al.*, 2019). This signaling pathway from the periphery to the brain needs to be further investigated e.g. in *in vitro* and *in vivo* trials examining the possible signaling of peripheral AMPK activity to the brain by the stimulation of afferent vagal fibers or by other mechanisms.

The concept of energy turnover as used in this thesis comprises the level of energy expenditure due to physical activity and the corresponding energy intake. In contrast, one could argue that inactive obese individuals also have a high energy turnover simply because they have an increased energy expenditure due to enhanced body mass and thus an increased energy requirement. But neither this nor an increased energy expenditure and correspondingly enhanced energy requirement due to pathological conditions represents the concept of energy turnover used in this thesis. The currently used energy turnover approach comprises enhanced energy expenditure solely due to physical activity. Since people have different fitness levels the intensity and duration of physical activity needed to achieve the same energy turnover may vary depending on individual resting energy expenditure. Therefore, in this thesis energy expenditure was described using the PAL and thus adjusting for differences in individual resting energy expenditure. Accordingly, the current used approach can be specified as 'metabolic energy turnover'.

In conclusion, a high meal frequency – especially when many small meals or liquid calories are consumed – is a risk factor for weight gain as the appetite control system is not able to adequately detect those small amounts of calories and to respond with a reduced energy intake at the following meal. Therefore, snacking should be avoided and sugar containing beverages like orange juice should be consumed with the meal instead of in-between meals (Chapter II).

Inactivity – especially when a Western Diet is consumed – is a risk factor for weight gain. In contrast, an increased energy turnover by low intensity physical activity improves appetite control independent from energy balance. The beneficial effect of physical activity on energy balance is therefore not solely explained by burning up more calories but by an improved appetite control resulting in an energy intake closely matching energy expenditure.

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Chapter V:

Discussion of Methods

This thesis comprises nutritional interventions measuring energy intake and energy balance during free-living conditions (Chapter II) and during strictly controlled metabolic chamber conditions (Chapter III). Although the outcome parameters were chosen carefully there are some methodological limitations that need to be addressed.

Assessment of energy intake

Since energy intake shows large fluctuations from day to day (Racette *et al.*, 2008; Schoeller, 2014) the short-term appetite control is of special interest and was investigated in the present nutritional intervention (Chapter III) over 24 hours during different energy turnover and energy balance conditions. Energy intake can be assessed by measuring acute *ad libitum* food intake during a single test meal or during 24 hours which was used during the *ad libitum* energy intake condition in Chapter III. In most cases, the energy consumed during *ad libitum* feeding periods is calculated using the nutrient contents provided by the manufacturer or by national databases. These values

are means and can vary with the food item actually consumed by the study participants. For example, it was shown that gross energy content of infant formulas measured by bomb calorimetry is 12% higher than energy content claimed by the manufacturer (Curtis *et al.*, 1986). Another problem arises when heterogeneous food items are not consumed in total and for example the edge of a pizza is left over. In such cases the macronutrient and energy content may differ between the total food item and the parts of the actually consumed food item. Using an *ad libitum* feeding protocol thus requires reliance on mean values for the consumed energy and nutrient content provided by manufacturers or national databases. Otherwise as for example used in a study by Stubbs *et al.* (2004), only homogeneous foods and own analyses of the provided food items by bomb calorimetry are needed (Stubbs *et al.*, 2004). In the present thesis, nutrient contents provided by manufacturers or national databases were used to calculate energy and macronutrient intake indicating possible minor inaccuracies in energy intake data.

In many studies that investigated energy intake *ad libitum* buffet test meals were performed since subjects can choose what to eat and the preference for special types of food can be assessed (e.g. sweet vs. salty foods). On the other hand, a buffet setting can lead to overconsumption because of the desire to try everything, because preferred foods are offered or as a consequence of occasional eating, when foods are provided that are liked very much but are normally rarely consumed by the subjects. Also the opposite effect can occur when mainly food is provided that is disliked and the subjects consume less than they would need to feel comfortably full. Thus, both can lead to high inter-individual variance in food intake during an *ad libitum* intake period and in many studies it is not reported whether such considerations were taken into account during the planning of the investigation. Nevertheless, there are also some trials where the authors addressed these potential issues, for example Thivel *et al.* (2018) asked the participants for their food preferences using a questionnaire and the top-rated items as well as items rated as 'liked but rarely consumed' were excluded from the buffet to avoid overconsumption and occasional consumption (Thivel *et al.*, 2018). Long *et al.* (2002) and Martins *et al.* (2007) asked participants to rank various buffet food items in order of preference and offered sandwich fillings and food types according to the second and

third choice during buffet test meals (Long *et al.*, 2002; Martins *et al.*, 2007). Others ensured acceptability of the provided food items and did not include subjects who disliked more than 4 items of the provided buffet meal (King *et al.*, 2010). In the study under strictly controlled metabolic chamber conditions included in this thesis (Chapter III), no buffet meal setting but a fixed diet was used during *ad libitum* energy intake condition. Subjects had limited options to choose between similar food items with comparable energy and macronutrient content (e.g. 1 out of 3 pizzas, 1 out of 3 pasta sauces, etc.) according to individual preferences. Only subjects who confirmed to eat all food items or at least one of the options were included in the study to avoid restraint eating. The same food items were provided on all intervention days to achieve comparable conditions and participants were served readily composed meals on plates (larger portions than required). To maintain constant macronutrient consumption during *ad libitum* energy intake participants were instructed to eat proportionally from every food item per meal until they feel comfortably full. Therefore, typical buffet meal limitations had no impact on energy intake in the present thesis but also differences in preference of single food items could not be evaluated. Another issue in measuring energy intake is that people mostly stop eating once their plate is empty (Gibbons *et al.*, 2019). Therefore, it can be assumed that participants were affected by the meal presentation and always just stopped eating once their plate was empty. However, the data showed that only one subject behaved this way and consumed all provided foods independent of energy turnover. Four other subjects consumed all provided foods during low and medium energy turnover but not with high energy turnover. Consequently, no general impact of the meal presentation on the *ad libitum* energy intake is assumed. Nevertheless, maintaining the fixed macronutrient balance and avoiding inaccuracies due to incomplete consumption of heterogeneous food items could have been prevented using a homogenous diet.

Probable prospective energy intake and appetite control can also be assessed indirectly by using visual analogue scales to investigate subjective appetite sensations. Visual analogue scales are commonly used due to the easy handling (pen & paper method or digital version) and the broad range of possible sensations that can be assessed (e.g. hunger, fullness, desire to eat, satiety, food preferences) (Flint *et al.*, 2000; Stubbs *et al.*,

2000). Since subjective feelings are investigated a large inter-individual variation must be expected as well as human error in completing the visual analogue scales (Stubbs *et al.*, 2000). However, in a study with duplicate measurements of hunger, satiety, fullness, prospective food consumption, desire to eat something sweet, salty, fatty or savoury and palatability of the meals using visual analogue scales under exactly the same conditions in 55 healthy men it was shown that visual analogue scales are a reproducible and valid tool to measure changes in subjective appetite sensations as indicated by the coefficient of repeatability (Flint *et al.*, 2000). Furthermore, it was shown that visual analogue scales are sensitive to experimental manipulations as alterations in diet composition or energy intake (Stubbs *et al.*, 2000). They show better reproducibility under controlled conditions compared to free-living conditions and should be used in a within-subject or cross-over design to minimize inter-individual variations and increase reproducibility (Stubbs *et al.*, 2000). Since both aspects of controlled conditions and cross-over design were fulfilled in the intervention included in this thesis (Chapter III) a high reproducibility of visual analogue scale ratings within each subject is anticipated. Ratings for desire of special food items are less reproducible (Flint *et al.*, 2000), indicating that relatively higher sample sizes are needed to detect significant differences for those questions. Thus, it is not surprising that no consistent results were found for food preferences among the subsample of 8 participants asked for their desire for something sweet, salty and fatty in the present thesis. To reliably assess such preferences, a minimum sample size of 16 subjects would have been needed.

Appetite control can also be investigated indirectly by measuring gastrointestinal peptide hormones which are known to influence energy intake. Ghrelin as orexigenic and GLP-1 as anorexigenic hormone were measured in the present thesis (Chapter III) because they are involved in short-term appetite control. Insulin concentrations were assessed since it has been shown that insulin, so far thought of as long-term signal of energy balance, is also associated with short-term appetite control in healthy subjects and that the postprandial insulin response may be an important satiety signal (Flint *et al.*, 2007). Furthermore, insulin has an impact on ghrelin concentrations (Murdolo *et al.*, 2003) and is influenced by GLP-1 levels (Kreymann *et al.*, 1987) making it an interesting

link between these peptides. Plasma samples were collected in tubes containing a mixture of protease, esterase and DPP-IV inhibitors to prevent hormonal degradation. In the current trial (Chapter III), total GLP-1 was assessed as marker of secretion. This is favorable compared to measuring active GLP-1_{7-36amid} since the active form is rapidly inactivated by DPP-IV before entering the circulation (Hansen *et al.*, 1999) as well as afterwards (Vilsbøll *et al.*, 2003) and since also inactivated GLP-1 found in the blood has been shown to previously stimulate vagal afferent fibers before its inactivation (Nakagawa *et al.*, 2004). GLP-1 is not only involved in appetite control but has several other biological functions. It is a major incretin hormone enhancing insulin secretion and reducing glucagon secretion in the postprandial phase (Kreymann *et al.*, 1987). This function can enhance the satiating effect of GLP-1 because insulin itself is an anorexigenic hormone that suppresses prandial ghrelin secretion (Murdolo *et al.*, 2003). Together with PYY, GLP-1 mediates the 'ileal brake effect', the feedback inhibition of proximal gastrointestinal motility and secretion caused by the presence of unabsorbed nutrients in the ileum (for review see (Maljaars *et al.*, 2008)). In this context, GLP-1 also reduces gastric acid secretion (O'Halloran *et al.*, 1990) and gastric emptying (Nauck *et al.*, 1997). The 'ileal brake' thus contributes to enhanced satiety and reduced food intake due to e.g. extended gastric distention (Geliebter, 1988; Welch *et al.*, 1988). Moreover, GLP-1 reduces blood pressure due to vasodilatation, enhances cardio protection, reduces inflammation in adipose and other tissues, enhances natriuresis and diuresis in the kidneys and reduces platelet coagulation (for review see (Drucker, 2016)).

Ghrelin secretion was assessed by measuring total ghrelin (Chapter III). This may mask relevant changes in acylated ghrelin which seem to be essential for the orexigenic effect (King *et al.*, 2010). But total ghrelin is assumed as appropriate surrogate since the ratio of acylated and total ghrelin is relatively robust (Cummings *et al.*, 2004) and in a within subject design it has been shown that total ghrelin is appropriately responsive to short-term energy balance perturbations (Hagobian *et al.*, 2008). Besides acting as orexigenic hormone in the hypothalamus ghrelin has various other biological functions (for review see (Korbonits *et al.*, 2004; Higgins *et al.*, 2007)). Ghrelin has important hormonal effects including stimulation of the release of growth hormone, adrenocorticotropin, cortisol and

aldosterone (Arvat *et al.*, 2001) as well as suppressing the secretion of testosterone (Tena-Sempere, 2005) and luteinizing hormone as shown in rhesus monkeys (Vulliémoz *et al.*, 2004). In rats it was demonstrated that ghrelin enhances gastric acid secretion and gastrointestinal motility (Masuda *et al.*, 2000) and reduces colonic transit time after central injection (Tebbe *et al.*, 2005) which may contribute to increased feelings of hunger. Ghrelin also has some cardio protective functions as it increases the stroke volume (Nagaya *et al.*, 2001) and reduces blood pressure due to systemic vasodilatation (Okumura *et al.*, 2002). It effects the autonomous nervous system by reducing renal sympathetic activity as shown in rabbits (Matsumura *et al.*, 2002) and by reducing serotonin release as shown in rats (Brunetti *et al.*, 2002). In addition, ghrelin has anti-inflammatory effects as it reduces the expression of various inflammatory cytokines (Dixit *et al.*, 2004) and the proliferation of T-cells in mice (Xia *et al.*, 2004). Furthermore, ghrelin has been shown to promote bone formation and to increase bone mineral density in rats (Fukushima *et al.*, 2004) as well as to improve sleep in humans (Weikel *et al.*, 2003).

It can be argued that gastrointestinal peptide hormones are not only involved in appetite control but also have other local and systemic functions. Thus, gastrointestinal peptide hormones themselves should not solely be seen as mediators of changes in appetite. On the other hand, the causality between higher ghrelin levels and hunger or food intake has been demonstrated after ghrelin compared to saline injection and following *ad libitum* buffet meal in 9 healthy adults (Wren *et al.*, 2001). It has also been shown that ghrelin administration and fasting have similar acute stimulatory effects on hedonic responses during food evaluations as measured with functional magnetic resonance imaging in healthy adults (Goldstone *et al.*, 2014). The causality between higher GLP-1 levels and reduced hunger or energy intake was demonstrated following the injection of GLP-1 compared to saline infusion in 20 healthy normal weight men (Flint *et al.*, 1998) and was confirmed using physiological doses of GLP-1 in obese men (Flint *et al.*, 2001). Furthermore, in triple-blind placebo-controlled trials in more than 200 subjects it was observed that the treatment of type 2 diabetes with the GLP-1 receptor agonist exendin-4 results in progressive weight loss (Buse *et al.*, 2004; DeFronzo *et al.*, 2005). In

conclusion, the role of gastrointestinal peptide hormones mediating appetite control is important although it is not the only function of these hormones.

Assessment of energy balance

In the nutritional intervention study (Chapter III), daily routine was strictly controlled and whole room calorimetry was used to measure exact 24h energy expenditure. Also energy intake was strictly controlled over the course of this trial because every single food item was weighed to the nearest 0.1 g for each subject. High quality energy balance data were therefore obtained. However, the method of whole room calorimetry has also some limitations: Only short-term measurements of 24 hours up to several days can be performed because the subjects have to stay in the metabolic chamber during the whole measurement period. Due to the limited space inside the metabolic chamber (9 m²) subjects are obliged to be relatively inactive aside from treadmill activities. This is not comparable to free-living daily life behavior since the overall conditions are more laboratory-like and may influence behavior inside the metabolic chamber. Therefore, especially when concerning behavior as energy intake or physical activity, acute results from metabolic chamber studies cannot automatically be transferred to long-term free-living conditions.

Long-term effects can be assessed indirectly by measuring changes in body composition, namely changes in fat mass and fat-free mass. This is possible since according to the first law of thermodynamics, which states that energy can be transformed but not created or destroyed, changes in energy balance lead to changes in energy stores and therefore changes in body composition (Hall *et al.*, 2012). Thus with assessing changes in body composition the effects of medium to long-term interventions on energy balance and appetite control can easily be measured as conducted in the free-living study included in this thesis (Chapter II). An advantage of this indirect approach is the opportunity to determine effects of free-living interventions on energy balance with relatively simple, non-invasive and inexpensive methods and without restrictions for the subjects comparable to metabolic chamber studies. In contrast, short-term changes in appetite control cannot be examined reliable with this approach since body weight in the

short-term fluctuates unrelated to changes in energy stores because of changes in hydration and gastrointestinal tract content (Hall *et al.*, 2012). In addition, devices for measurement of body composition may not be able to detect such small changes in body composition thus the minimal detectable change (MDC) of a certain method must be taken into account. Air-displacement plethysmography was used in the orange juice trial (Chapter II) to measure changes in body composition before and after two weeks of intervention. Since participants should maintain their usual eating and activity habits only minor changes in body composition were expected. Therefore, it was important to reduce the minimal detectable change of air-displacement plethysmography to be able to reliably detect those minor changes. The minimal detectable change is equal to the smallest real change, i.e. the amount by which the fat mass of a subject needs to change to be sure that the change is greater than the measurement error (Bosy-Westphal & Müller, 2014). It can be interpreted as the magnitude of change below which there is more than 95% chance that no real change has occurred and is calculated as $MDC_{95} = 1.96 \times \sqrt{z} \times SEM$ with z , the number of repeated measurements conducted and with SEM, the standard error of the mean between the repeated measurements (Bacon & Aphramor, 2011). To improve the precision of air-displacement plethysmography all measurements were conducted in duplicate leading to a reduced minimal detectable change of 0.53 kg fat mass compared to 0.72 kg fat mass with single within day measurements (calculated with data of an in-house repeated measurement study). Thus, also small changes of only 0.53 kg in fat mass after two weeks of orange juice consumption could be reliably assessed in the present study (Chapter II).

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Summary

Over the last decades, consumption of liquid calories as sugar containing beverages like fruit juices, soft drinks and sweetened coffees increased and became a major risk factor for weight gain. The aim of the first study was to investigate if orange juice consumption together with meals improves energy balance compared to juice consumption in-between meals. The impact of consuming 20% of individual daily energy requirement as orange juice with or in-between three meals per day on weight gain and body composition was therefore investigated in a 2 x 2-week crossover intervention under free-living conditions in 26 healthy adults. Despite the same energy intake in both interventions only the consumption of orange juice in-between meals resulted in a gain in fat mass. This discrepancy seems to be explained by a lack in compensation of energy intake at the following meal when orange juice is consumed in-between meals compared to juice consumption with meals. In conclusion, consumption of liquid calories in-between meals is a risk factor for weight gain and thus snacking should be avoided. In contrast, consumption of sugar containing beverages together with only three meals per day was able to prevent weight gain.

Beyond liquid calories, a low energy turnover may also be a risk factor for weight gain. In line with this hypothesis it was proposed that weight control is improved when physical activity and energy intake are both high (high energy turnover) but not with sedentary behavior. In a second study, the acute impact of energy turnover on appetite control was therefore investigated in 16 healthy adults under strictly controlled conditions in a metabolic chamber at different levels of energy balance. Three levels of 24h energy turnover (low PAL 1.31; medium PAL 1.55; high PAL 1.76) were compared in a randomized crossover intervention each under *ad libitum* energy intake, zero energy balance, caloric restriction (-25%) and overfeeding (+25%). Different levels of energy turnover were achieved by varying durations of low intensity physical activity on a treadmill (low 0 min; medium 3 x 55 min; high 3 x 110 min). Independent from energy balance, appetite control was improved with high energy turnover as shown by lower ghrelin and higher GLP-1 concentrations, correspondingly reduced hunger and desire to eat and an equal energy balance during *ad libitum* energy intake. Thus, inactivity is a

risk factor for weight gain and low intensity physical activity can be sufficient to maintain energy balance as long as a physical activity level of 1.76 is reached. Accordingly, the beneficial effect of physical activity on energy balance is not solely explained by burning up more calories but also by an improved appetite control resulting in an energy intake closely matching energy expenditure.

Zusammenfassung

Der Verzehr kalorienhaltiger Getränke wie Fruchtsaft, Limonade und gesüßte Kaffeegetränke hat in den letzten Jahrzehnten zugenommen und ist zu einem Hauptrisikofaktor für Gewichtszunahme geworden. Ziel der ersten Studie war es zu untersuchen, ob der Verzehr von Orangensaft zusammen mit der Mahlzeit die Energiebilanz im Vergleich zum Verzehr zwischen den Mahlzeiten verbessert. Hierfür wurde in einer 2 x 2-Wochen *Crossover*-Studie bei 26 gesunden Erwachsenen der Einfluss von 20% des individuellen Tagesenergiebedarfs in Form von Orangensaft zusammen mit oder zwischen drei Mahlzeiten pro Tag auf die Gewichtszunahme und Körperzusammensetzung untersucht. Trotz der gleichen Energieaufnahme in Form von Orangensaft in beiden Interventionen führte nur der Verzehr zwischen den Mahlzeiten zu einer Zunahme an Körperfettmasse. Ursächlich für diese Diskrepanz könnte eine fehlende Kompensation der Energieaufnahme in der Folgemahlzeit bei Konsum von Orangensaft zwischen den Mahlzeiten sein. Zusammenfassend ist der Verzehr von kalorienhaltigen Getränken zwischen den Mahlzeiten ein Risikofaktor für Gewichtszunahme und *Snacking* sollte daher vermieden werden. Im Gegensatz dazu konnte eine Gewichtszunahme durch den Verzehr von zuckerhaltigen Getränken zusammen mit nur drei Mahlzeiten am Tag verhindert werden.

Neben kalorienhaltigen Getränken könnte auch ein niedriger Energieumsatz ein Risikofaktor für eine Gewichtszunahme sein. Diese Hypothese besagt, dass die Gewichtskontrolle verbessert ist, wenn körperliche Aktivität und Energieaufnahme beide hoch sind (hoher Energieumsatz). In einer zweiten Studie wurde daher der Einfluss von kurzfristigen Veränderungen des Energieumsatzes auf die Appetitkontrolle bei 16 gesunden Erwachsenen unter streng kontrollierten Bedingungen in einem Stoffwechselraum während verschiedener Energiebilanzen untersucht. Jeweils drei Tage mit unterschiedlichem Energieumsatz (niedrig PAL 1,31; mittel PAL 1,55; hoch PAL 1,76) wurden in einer randomisierten Intervention im *Crossover*-Design unter *ad libitum* Energieaufnahme, ausgeglichener Energiebilanz, Kalorienrestriktion (-25%) und Überernährung (+25%) untersucht. Die verschiedenen Energieumsätze wurden durch eine unterschiedliche Dauer von körperlicher Aktivität auf einem Laufband (niedrig 0 min;

mittel 3 x 55 min; hoch 3 x 110 min) bei niedriger Intensität erreicht. Tatsächlich konnte gezeigt werden, dass die Appetitkontrolle bei hohem Energieumsatz unabhängig von der Energiebilanz verbessert war. Dies wurde durch geringere Ghrelin- und höhere GLP-1-Spiegel, geringeren Hunger und Appetit und eine ausgeglichene Energiebilanz bei *ad libitum* Energieaufnahme während eines hohen Energieumsatzes gezeigt. Folglich kann körperliche Aktivität mit niedriger Intensität ausreichend sein, um eine ausgeglichene Energiebilanz aufrecht zu erhalten solange ein *Physical Activity Level* von 1,76 erreicht wird. Demnach ist der vorteilhafte Effekt von körperlicher Aktivität auf die Energiebilanz nicht allein durch den erhöhten Energieverbrauch zu erklären, sondern auch durch eine bessere Anpassung des Appetits an einen höheren Energiebedarf.

Appendix

Ethics committee vote | Orange Juice Study

Informed consent form | Orange Juice Study

Confirmation of Co-authorship | Orange Juice Study

Ethics committee vote | Energy Turnover Study

Informed consent form | Energy Turnover Study

Visual Analogue Scales | Energy Turnover Study

Confirmation of Co-authorship | Energy Turnover Study

Curriculum Vitae

Danksagung

LANDESÄRZTEKAMMER BADEN-WÜRTTEMBERG
KÖRPERSCHAFT DES ÖFFENTLICHEN RECHTS

ETHIK-KOMMISSION



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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

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

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

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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 Anna Hägele

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

High orange juice consumption with or in-between three meals a day differently affects energy balance 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
Concept: 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
Planning: 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
Execution: Involvement in the analysis or the concrete experiments/investigation	C
Manuscript preparation: Presentation, interpretation and discussion of the results obtained in article form	C

4. Signature of all co-authors

Date	Name	Signature
10.6.2019	Anja Bosy-Westphal	
22.6.2019	Reinhold Carle	
1.7.2019	Ralf Schweiggert	
16.08.2019	Julian Aschoff	
29.05.2019	Franziska Büsing	F. Büsing
30.07.2019	Alessa Nas	A. Nas

19.06.19	Lena Gnädinger	
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5. Signature of doctoral candidate		
Date	Name	Signature
20.08.19	Franziska Anna Hägele	

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)
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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:

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- 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

A handwritten signature in blue ink, appearing to read 'G. Hook'.

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

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.

Name des Teilnehmers (Druckbuchstaben)

Unterschrift des Teilnehmers

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. oec. troph. Dr. med. Anja Bosity-
Westphal

Name des aufklärenden Arztes

Unterschrift der aufklärenden Person des Studienpersonals

Datum, Ort

VASx.1 Proband: _____ Datum: _____ Kodierung: _____ Stand 25.06.2017

Bitte markiere mit einem **senkrechten Strich** auf der Linie wie stark du das jeweilige Gefühl **gerade jetzt** empfindest. Dabei ist es wichtig dein tatsächliches Gefühl zu markieren und nicht ein Gefühl von dem du denkst es wäre jetzt angebracht. Bitte nutze die komplette Breite der Linie und betrachte die beiden Enden dabei als Extreme – so wenig / stark wie du das Gefühl vorher noch nie empfunden hast.

Wie **hungrig** fühlst du dich jetzt gerade?

überhaupt nicht |-----| extrem

Wie **voll** fühlst du dich jetzt gerade?

überhaupt nicht |-----| extrem

Wie groß ist dein **Verlangen etwas zu essen** jetzt gerade?

überhaupt nicht |-----| extrem

VASx.1 Proband: _____ Datum: _____ Kodierung: _____ Stand 25.06.2017

Bitte markiere mit einem **senkrechten Strich** auf der Linie wie stark du das jeweilige Gefühl **gerade jetzt** empfindest. Dabei ist es wichtig dein tatsächliches Gefühl zu markieren und nicht ein Gefühl von dem du denkst es wäre jetzt angebracht. Bitte nutze die komplette Breite der Linie und betrachte die beiden Enden dabei als Extreme – so wenig / stark wie du das Gefühl vorher noch nie empfunden hast.

Würdest du gerne etwas **süßes** essen?

Nein, überhaupt nicht | Ja, sehr gerne

Würdest du gerne etwas **salziges** essen?

Nein, überhaupt nicht | Ja, sehr gerne

Würdest du gerne etwas **fettiges** essen?

Nein, überhaupt nicht | Ja, sehr gerne

C A U	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 Anna Hägele

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

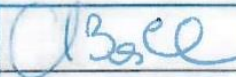
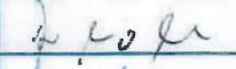

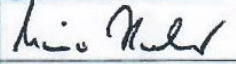
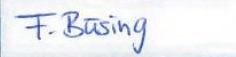
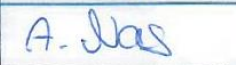
Appetite control is improved by acute increases in energy turnover at different levels of energy balance

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%)

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Planning: 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
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Manuscript preparation: Presentation, interpretation and discussion of the results obtained in article form	C

4. Signature of all co-authors

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25/7/19	John Edward Blundell	
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-

ORIGINALARBEITEN

Hägele FA, Büsing F, Nas A, Hasler M, Müller MJ, Blundell JE, Bosy-Westphal A; Appetite control is improved by acute increases in energy turnover at different levels of energy balance; *The Journal of Clinical Endocrinology & Metabolism* 2019, 104(10):4481-4491; DOI: 10.1210/jc.2019-01164

Nas A, Büsing F, **Hägele FA**, Hasler M, Müller MJ, Bosy-Westphal A; Impact of energy turnover on fat balance in healthy adults during energy balance, caloric restriction and overfeeding; *British Journal of Nutrition* 2019; *under revision*

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Nas A, Mirza N, **Hägele F**, Kahlhöfer J, Keller J, Rising R, Kufer TA, Bosy-Westphal A; Impact of breakfast skipping compared with dinner skipping on regulation of energy balance and metabolic risk; *American Journal of Clinical Nutrition* 2017, 105(6):1351-61; DOI: 10.3945/ajcn.116.151332

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KONGRESS- UND TAGUNGSBEITRÄGE

Hägele FA, Büsing F, Nas A, Bosity-Westphal A; Einfluss des Energy Flux auf die Appetitregulation; 69. *Öffentliche Hochschultagung der Agrar- und Ernährungswissenschaftlichen Fakultät der Christian-Albrechts-Universität zu Kiel*, 2019 – Kongressvortrag

Hägele FA, Büsing F, Nas A, Bosity-Westphal A; Einfluss des *Energy Flux* auf die Appetithormonspiegel bei gesunden Probanden; 12. *Diabetes Herbsttagung der DDG & 34. Jahrestagung der DAG*, Wiesbaden, 2018 – Posterpräsentation

Hägele FA, Büsing F, Nas A, Bosity-Westphal A; Impact of energy flux on levels of appetite hormones in healthy subjects; *International Congress on Obesity and Metabolic Syndrome 2018*, Seoul, Südkorea – Kongressvortrag

Hägele FA, Büsing F, Nas A, Bosity-Westphal A; Impact of energy flux on subjective appetite sensations and *ad libitum* energy intake in healthy subjects; 25. *European Congress on Obesity*, Wien, Österreich, 2018 – Posterpräsentation

Hägele FA, Büsing F, Nas A, Bosity-Westphal A; Impact of energy flux on subjective appetite during energy balance, caloric restriction and overfeeding in healthy subjects; 55. *Kongress der Deutschen Gesellschaft für Ernährung e.V.*, Hohenheim, Stuttgart, 2018 – Kongressvortrag

Hägele FA, Büsing F, Nas A, Bosity-Westphal A; Impact of energy flux on subjective appetite during energy balance, caloric restriction and overfeeding in healthy subjects; *Recent Advances and Controversies in the Measurement of Energy Metabolism*, Fribourg, Schweiz, 2017 – Posterpräsentation

Hägele F, Büsing F, Nas A, Bosity-Westphal A; Impact of regular orange juice consumption with or in-between meals on glucose regulation in healthy subjects; 24. *European Congress on Obesity*, Porto, Portugal, 2017 – Posterpräsentation

Hägele F, Keller J, Nas A, Kahlhöfer J, Bosity-Westphal A; Einfluss von Meal Skipping bei ausgeglichener Energiebilanz auf die Tagesglykämie und den postprandialen Glukosestoffwechsel bei gesunden Probanden; 54. *Kongress der Deutschen Gesellschaft für Ernährung e.V.*, Kiel, 2017 – Kongressvortrag

Hägele F, Karschin J, Kahlhöfer J, Nas A, Bosity-Westphal A; Einfluss von Meal Skipping bei ausgeglichener Energiebilanz auf die Tagesglykämie und den postprandialen Glukosestoffwechsel bei gesunden Probanden; *Ernährung 2016*, Dresden – Kongressvortrag

Hägele F, Karschin J, Kahlhöfer J, Nas A, Bosity-Westphal A; Impact of the timing of meal skipping on postprandial glucose metabolism and daylong glycaemia in healthy subjects; 23. *European Obesity Summit*, Göteborg, Schweden, 2016 – Posterpräsentation

AUSZEICHNUNG & STIPENDIEN

- 2018 **Reisestipendium** des Kongresskomitees des *International Congress on Obesity and Metabolic Syndrome 2018* für die Präsentation eigener Forschungsergebnisse, Seoul, Südkorea
- 2018 **Reisestipendium** von Danone GmbH Deutschland für die Präsentation eigener Forschungsergebnisse auf dem *25. European Congress on Obesity 2018*, Wien, Österreich
- 2016 **Abstract-Preis** der Deutschen Gesellschaft für Ernährungsmedizin e.V. für den Beitrag zum Kongress *Ernährung 2016*, Dresden

Danksagung

Ich danke...

... Prof. Dr. Dr. Anja Bosy-Westphal für die Möglichkeit meine Doktorarbeit in Ihrer Arbeitsgruppe schreiben zu dürfen und den damit verbundenen Einstieg in die Welt der Humanstudien. Vielen Dank für jeden Rat, die interessanten wissenschaftlichen Gespräche und den Einblick in aktuelle Forschungsfragen. Danke für dein Vertrauen, dass du von Anfang an in mich gesetzt hast und deine unermüdliche Power, die einfach ansteckend ist. Du hast mich stets in hohem Maß gefordert und gefördert, wodurch ich fachlich und persönlich über mich hinauswachsen und mich immer weiter verbessern konnte. Danke auch für die Möglichkeit so viele nationale und internationale Kongresse mit dir besuchen zu dürfen und meine Ergebnisse von Anfang an zu präsentieren. Diese Erfahrungen waren sehr bereichernd und die Reisen immer ein Erlebnis. Danke für das unermüdliche Korrekturlesen von Manuskripten, Abstracts, Postern und Vorträgen zu jeder Tages- und Nachtzeit. Während der letzten 4 Jahre durfte ich unendlich viel von dir lernen und ich bin sehr froh auch weiterhin mit dir zusammenarbeiten zu können.

... Dr. Franziska Büsing für fast 4 Jahre unübertroffene Teamarbeit! Du warst mir die beste Kollegin und Freundin, die ich mir für die Promotion wünschen konnte. Als Franzis² waren wir ein unschlagbares Team, ich konnte mich in allen Lebenslagen blind auf dich verlassen und gemeinsam haben wir jede kleinere oder größere Katastrophe gemeistert. Ohne dich an meiner Seite wäre es mir deutlich schwerer gefallen nach Kiel zu gehen. Mein Dank an dich lässt sich nicht in Worte fassen und geht weit über gemeinsame Nacht- und Wochenendschichten, Kongressreisen, Fortbildungen, Feiern und Sinnkrisen hinaus. Es wird unglaublich schwer in Zukunft ohne dich zu arbeiten!

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DANKE!