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MASTER THESIS

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One-carbon metabolites and markers of B-vitamin status as predictors of inter-individual weight loss response to diets with different fat and carbohydrate content

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Abstract

Background: The prevalence of overweight and obesity is increasing, and it has been estimated that 39% of the world's population is above normal weight. This is worrisome as both overweight and obesity is causally linked to a wide range of chronic diseases and effective strategies to achieve and maintain weight loss are therefore needed. In order to lose weight, one has to be in a negative energy balance over time. This can be achieved through a variety of diets, but no single strategy has been shown to be superior on average. However, substantial inter-individual variation in the weight loss response to different diets has been demonstrated. Identifying biological factors which may predict who will respond to a given diet therefore has the potential to improve our understanding and treatment of overweight and obesity.

The peroxisome proliferator-activated receptor α (PPARα) is an important regulator of energy metabolism and specifically increases the metabolism of fat upon activation. Thus, endogenous PPARα-activity may be a key factor influencing the response to diets differing in fat content. The aim of this study was therefore to determine whether baseline plasma concentrations of metabolites related to one-carbon metabolism and B-vitamin status, of which some have been suggested as potential biomarkers of PPARα-activity, was associated with weight loss and moreover could predict inter-individual differences in the weight loss response to two diets with very different fat- and carbohydrate content. We further hypothesized that the metabolites suggested to reflect endogenous PPARα-activity would be similarly associated with weight loss response to the diets.

Methods: For a period of 12 weeks, 46 men with obesity between the ages of 30-50 years old were randomly allocated to either following a low-fat, high-carbohydrate diet (LFHC) or a very-high fat, low-carbohydrate diet (VHFLC). Both diets were equal in terms of protein and energy content. Measurements of body weight as well as fasting blood samples were collected at baseline and every study visit. Baseline plasma metabolite concentrations were determined by gas- and liquid chromatography coupled to tandem mass spectrometry or microbiological assay. The overall association between baseline metabolite concentrations and subsequent weight loss were assessed by calculating Spearman correlations with weight change in % at 12 weeks. To visualize the association between baseline metabolite concentration and weight loss, weight loss in % from baseline to 12 weeks was plotted against the metabolite

concentrations at baseline. The individuals were coloured according to diet group, and linear regression lines extrapolated to the range of the metabolites were superimposed on the plot to visualize trends within the diet groups. To formally test the potential interaction between the baseline metabolite concentrations and the diets, an interaction term was added to a linear regression analysis comparing body weight at week 12, adjusted for baseline body weight.

Results: We did not observe strong associations between baseline plasma metabolite concentrations and weight loss. However, we did observe some differential trends in weight loss response according to baseline plasma metabolite concentrations and diet type. Specifically, we observed that the LFHC-diet outperformed the VHFLC-diet for lower baseline concentrations of homocysteine, sarcosine, N1-methylnicotinamide (mNAM), pyridoxal (PL), pyridoxal-5-phospate (PLP), 4-pyridoxic acid (PA) and methylmalonic acid (MMA) and for higher concentrations of cystathionine, betaine, dimethylglycine (DMG), glycine and folate. The VHFLC-diet on the other hand was observed to produce the superior result when concentrations of glycine were lower and for higher concentrations of homocysteine, sarcosine, mNAM, PL, PLP, PA and MMA. However, the formal tests for interaction between baseline metabolite concentrations and diet type were inconclusive. Of the metabolites previously suggested to reflect PPARα-activity, we observed that mNAM, PL, PLP and MMA demonstrated similar trends in terms of which diet was more effective according to higher or lower concentrations, but glycine and DMG demonstrated opposite trends.

Conclusion: In this study, metabolites related to one-carbon metabolism and markers of B-vitamin status at baseline was not strongly associated with weight loss. However, some differential trends were observed in the weight loss response to the different diets. Of the metabolites suggested to reflect PPARα-activity, somewhat conflicting associations were seen. Nevertheless, some of these metabolites may have potential in identifying individuals who would benefit from each diet and may therefore potentially be of value when considering individual dietary advice for weight loss. To further explore whether any of these metabolites can be used to tailor dietary weight loss advice to the individual, a first step should be to perform similar studies in other existing dietary intervention trials, preferably with larger sample sizes. From there, the most promising metabolites should ultimately be tested in randomized controlled trials to determine whether, and how, stratification by metabolites according to baseline concentration can be utilized for personalized nutrition.

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List of abbreviations

BHMT	Betaine-homocysteine methyltransferase
BMI	Body Mass Index
BMR	Basal metabolic rate
DIT	Diet-induced thermogenesis
DMG	Dimethylglycine
FAD	Flavin adenine dinucleotide
FMN	Flavin mononucleotide
GC-MS/MS	Gas chromatography – Tandem mass spectrometry
GNMT	Glycine N-methyltransferase
$HbA1_c$	Glycated haemoglobin
Holo-TC	Holo-transcobalamin
HOMA1-IR	Homeostatic model assessment of insulin resistance
ICC	Intraclass correlation coefficient
LC-MS/MS	Liquid chromatography - Tandem mass spectrometry
LFHC	Low-fat, high-carbohydrate diet
MMA	Methylmalonic acid
mNAM	N1-methylnicotinamide
MS	Methionine synthase
mTHF	5-methyltetrahydrofolate
MTHF	5,10-methylenetetrahydrofolate
MTHFD1	Methylenetetrahydrofolate dehydrogenase complex 1
MTHFR	Methylenetetrahydrofolate reductase
NA	Nicotinic acid

NAD	Nicotinamide adenine dinucleotide
NAM	Nicotinamide
PA	4-pyridoxic acid
PL	Pyridoxal
PLP	Pyridoxal-5-phosphate
PPAR	Peroxisome proliferator-activated receptor
PUFA	Polyunsaturated fatty acids
RER	Respiratory exchange ratio
SAH	S-Adenosylhomocysteine
SAM	S-Adenosylmethionine
SHMT	Serine hydroxymethyltransferase
THF	Tetrahydrofolate
VHFLC	Very-high fat, low-carbohydrate diet
WHO	World Health Organization

1 Introduction

1.1 Overweight and obesity

Globally, overweight and obesity has increased substantially during the past decades. In fact, between 1975 and 2016, the prevalence of obesity almost tripled. In 2016, 1.9 billion adults met the criteria of being overweight and of these about 650 million met the criteria of being obese, corresponding to 39% of the world's adult population being above normal weight (1). This has made the World Health Organization (WHO) recognize overweight and obesity as a global epidemic that poses an important public health challenge (2). Similarly, in Norway it has been estimated that 61.7 % of the adult population suffer from overweight whereof 23.1 % suffer from obesity (3). Both overweight and obesity can be defined as an excessive accumulation of fat that may impair health (1). The striking increase in people suffering from overweight and obesity is worrisome as both are associated with increased risk of other chronic diseases such as diabetes, cardiovascular diseases, different types of cancers and osteoarthritis (1). Moreover, 2.8 million annual deaths can be attributed to overweight and obesity (4). Overweight and obesity also impose a rather large economic burden as the global economic impact of obesity was estimated to be 2.0 trillion US dollars in 2014 (5). In light of the negative consequences of overweight and obesity, it is important to find effective strategies to reverse this negative development.

1.2 Measuring and classification of overweight and obesity

Overweight and obesity are most commonly defined by body mass index (BMI, kg/m^2). BMI is an easy tool to use for this purpose as it only requires the height and weight of a person to get an estimate of whether the person is underweight, normal weight, overweight or obese. WHO defines overweight as a BMI equal to or greater than 25, and obesity as a BMI equal to or greater than 30 (1). In addition to BMI, waist circumference is often used as an additional measure to assess whether an individual is at risk for metabolic syndrome, cardiovascular disease or diabetes type 2, and WHO has defined waist circumference cut-off points for the risk of developing these diseases (6-8). A waist circumference above 80 cm for women and 94 cm for men is associated with increased risk of developing diabetes type 2 and cardiovascular disease, and a waist circumference above 88 cm for women and 102 cm for men is associated with an even higher risk of developing these diseases (6). There are also other tools that can be used to identify overweight and obesity and to evaluate body

composition. These methods include double indirect methods such as skinfold measurements and bioelectric impedance analysis and indirect methods such as total body water, dual-energy X-ray absorptiometry, computed tomography and magnetic resonance imaging (9). However, several of these tools have limited use in overweight and obese individuals due to body size limitations related to the machines or the methods are not well enough developed to assess individuals with overweight and obesity (10).

1.3 Energy balance and the causes of overweight and obesity

1.3.1 Definition of energy balance and energy intake

When discussing overweight and obesity, the concept of energy balance is particularly relevant. Energy balance can be defined as "The physiological state in which daily energy intake equals energy expenditure and both body weight and energy content (defined by body composition) are constant" (7). Energy balance can thus be represented by an equation consisting of energy intake on the one side and energy expenditure on the other side and where imbalances in this equation over time will lead to changes in energy storages. The intake of energy comes from the food and drinks we consume which contains energy in the form of fat, carbohydrates including dietary fibre, protein and to a lesser extent alcohol (11). In the context of energy balance, energy intake more specifically refers to the amount of energy absorbed, not energy eaten. However, the difference between energy consumed and energy absorbed, due to incomplete digestion and absorption, is usually negligible (12).

1.3.2 Energy expenditure

The three main components that make up energy expenditure are basal metabolic rate (BMR), diet-induced thermogenesis (DIT) and physical activity (7). BMR is the energy cost of maintaining homeostasis and vital body functions while at rest and comprises roughly two-thirds of daily energy expenditure (7, 12). BMR is also positively correlated to body mass, and especially to the amount of fat-free mass (7, 11). Furthermore, energy is also required in order to digest, absorb and metabolize the foods consumed and this is referred to as DIT. The DIT of the different macronutrients vary, however it has been estimated that DIT comprises about 10% of daily energy expenditure in individuals who consume a varied diet and are in energy balance (7, 13). Energy expenditure from physical activity not only include physical exercise, but all bodily movements produced by skeletal muscle which results in the expenditure of energy (14). Physical exercise is a subcategory of physical activity and can be

defined as a planned and structured activity with a goal of maintaining or improving physical fitness or function (14). Nevertheless, physical activity is the component of energy expenditure that varies the most among individuals and to the greatest degree can be modified as it is highly dependent on a person's behaviour.

1.3.3 Energy storage

Energy balance is reflected in the body's energy storages and an imbalance between energy intake and energy expenditure over time ultimately leads to either an increase or a decrease in the body's total energy stores depending on whether the energy balance points in a positive or negative direction. The body energy stores refer to the energy reserves of fat, carbohydrate and protein stored in muscle- and fat tissue as well as in glycogen storages in the body. Glycogen can be referred to as our short-term storage of energy in the form of multibranched chains of glucose and is mainly stored in the liver (~100 g) and muscle tissue (~400 g) to provide easily accessed energy when needed (12, 15). However, the body has limited capacity to store energy in the form of glycogen. Thus, when energy intake exceeds energy expenditure over time, the excess energy beyond what the body can store as glycogen will therefore be stored as fat in adipose tissue (12). Adipose tissue is the body's largest energy storage and 1 kg of body fat contains to approximately 7000 kcal whereas 1 kg of muscle mass corresponds to about 1200 kcal (12). Although body weight may fluctuate independently of energy balance, primarily due to short-term fluctuations in hydration status, long term changes in the energy stores will ultimately lead to corresponding changes in body weight (12).

1.3.4 The causes to overweight and obesity

As described, overweight and obesity are caused by a positive energy balance over time. However, the underlying factors contributing to a positive energy balance are many and complex. One of the factors that has been highlighted as an important contributor is the global shift towards an increased availability, promotion and intake of energy dense foods with high contents of sugars and fats (1). Furthermore, reduced energy expenditure following a decrease in physical activity as a result of increased urbanization and less labour-intensive work, is another contributing factor, as being physically active has become more dependent on a person's intentional behaviour (1, 16). Studies have also investigated whether hormones related to appetite regulation may contribute to the development of overweight and obesity in

terms of driving excessive food intake. Findings indicate an association between obesity and reduced postprandial response of satiety hormones such as glucagon-like peptide 1 and peptide YY in addition to hormones promoting hunger such as ghrelin, which favours excessive energy intake (17). However, because most of the studies have measured the difference in these hormones between overweight or obese and normal weight individuals, it is not possible to distinguish whether postprandial reductions in these hormones contribute causally to the development of overweight and obesity or result from being overweight or obese (17). The role of genetics and their potential influence in the development of overweight and obesity has also been increasingly studied over the past years. Several obesity-related gene variants have been identified, however with the exception of a few rare single genetic variants causing monogenic obesity, it was reported that these gene variants were only able to explain about 6% of the variation in BMI in the general population until recently (18). However, recent studies report that the proportion of variation in BMI explained by genetic traits in the general population might be as high as 40% (19).

1.4 Negative health effects of overweight and obesity

Overweight and obesity can have negative health impacts and are causally linked to increased morbidity and mortality. About 70% of patients with diabetes type 2 are overweight, and the risk of developing diabetes type 2 increases with increasing overweight (20). Overweight is also associated with elevated blood lipids and hypertension, and thus increases the risk of developing cardiovascular diseases such as coronary heart disease (21, 22). Furthermore, overweight and obesity are also associated with conditions affecting the musculoskeletal system such as osteoarthritis which causes joint degeneration and consequently dysfunction and pain related to affected areas (23). Obesity has been found to be a significant risk factor for both the development and progression of osteoarthritis of the knee and a less strong association has been found between obesity and osteoarthritis of the hip. Proposed explanations include the increased mechanical stress to the joints due to increased body weight, as well as alterations in the growth and structure of tissues due to increased synthesis of endocrine factors following expansion of the adipose tissue (23).

Overweight may also increase the risk of certain types of cancer such as breast cancer, endometrial cancer, prostate cancer and cancers in the colon among others (24). The pathophysiology is not fully understood, but one possible underlying mechanism may be

related to the increased production of various hormones that may stimulate cell differentiation and growth. For example, people suffering from overweight often have elevated blood concentrations of insulin- and insulin-like growth factors and these hormones may help stimulate the development of cancer tumours (24). Furthermore, people with overweight often have a chronic low-grade inflammation which may also increase the risk of developing cancer (24). Overweight and obesity not only increase the risk of physical diseases but can also affect mental health. In this regard, overweight and obesity have been associated with low self-esteem, body-image dissatisfaction, depression and eating disorders such as binge-eating disorder among others (25, 26). Furthermore, the society's idealization of leanness and weight stigmatization towards overweight and obese individuals has been identified as contributing factors to the above mentioned damaging psychological consequences associated with overweight and obesity (27).

1.5 Health effects of weight loss

Weight loss is recommended for individuals with overweight and obesity to improve health and reduce the likelihood of developing obesity-related comorbidities. The goal however doesn't necessarily have to be to achieve what is defined as a normal weight or BMI. As a matter of fact, a modest weight loss of 5-10% of total body weight has been shown to be beneficial for individuals struggling with overweight and obesity as it improves metabolic health and reduces the risk of developing metabolic syndrome (28). A weight loss of 5-10% of initial body weight has also been found to improve cardiovascular risk factors such as glycated haemoglobin ($HbA1_c$), blood pressure and blood lipids among overweight and obese individuals (29). These findings indicate that encouraging individuals with overweight and obesity to lose 5-10% of their body weight may have profound health effects. In addition, it may be easier to motivate individuals to lose 5-10% of their body weight rather than striving to reach a BMI corresponding to normal weight.

1.6 Weight loss diets

In order for a diet to be effective for weight loss it has to lead to a negative energy balance over time and a variety of diets can be used to achieve this. Some diets limit certain macronutrients while other diets limit certain types of foods. Low-carbohydrate, high-fat diets such as the Atkins diet limit the intake of carbohydrates with focus on high energy intakes from fat (30). On the other hand, low-fat diets such as the DASH diet usually limits the

energy intake of fat to 20-35% of total energy intake and consists of higher amounts of carbohydrates (30). Very-low-fat diets further limit the intake of fat to under 20% of total energy intake, and examples are the Pritikin and Ornish diets (30). However, these diets ultimately work through the same mechanism, by creating an energy deficit, and no single strategy has been shown to be superior on average (31, 32). Moreover, long term weight loss maintenance has proven to be difficult irrespective of the type of diet as many dieters regain most of the lost weight within a short period of time (33-35). This is troublesome as it indicates that weight loss diets on average are not very effective in weight maintenance which should be the ultimate measure of weight loss success.

At the same time, solely focusing on average weight loss in dietary intervention studies might lead to a masking of the individual variability in response to dietary interventions (33). For example, a study investigating the effect of a low-fat diet compared to a low-carbohydrate diet found similar average weight loss effects in both groups, however there was a large inter-individual variation in the response to both diets with some participants losing 30 kg whereas others gained 10 kg during the study period (36). Similar inter-individual variations in weight loss response to diets differing in macronutrient content has also been reported in other dietary intervention studies (37, 38). Inter-individual differences in weight loss success can of course to a certain degree be attributed to individual differences in adherence to the diets, and higher self-reported dietary adherence has been associated with greater weight loss (39). On the other hand, there might be other factors influencing the weight loss response to dietary interventions such as individual differences in genetics, metabolism, gut microbiota or adaptive thermogenesis among others (40, 41). A better understanding and identification of individual characteristics or factors which may cause individuals to respond differently to dietary interventions can therefore have tremendous potential in terms of guiding individuals to choose the most effective weight loss strategy for them. Personalized nutrition is a field that seeks to identify such factors and the interest and research in this field of nutrition is increasing.

1.7 Personalized nutrition

The nutritional recommendations issued to the general population in today's society is to a certain extent based on the idea that one diet is healthiest and works best for all. However, this approach does not take into account the above mentioned often-observed inter-individual

variation in response to dietary interventions. Personalized nutrition on the other hand is an approach which seeks to tailor nutritional recommendations based on individual characteristics or traits. The personalization of nutritional recommendations and advice can however be viewed as occurring at different levels (42). One of the simplest forms of personalized nutrition is stratified nutrition where the general nutritional guidelines are tailored to subgroups in the population based on determinants such as gender or age (42). More individualized approaches of personalized nutrition are based on the addition of phenotypic information through for example anthropometrical measurements (BMI, waist circumference etc.), metabolic or biochemical analysis or measurements of physical activity (42). Another and perhaps the most individualized type of personalized nutrition is directed around the identification of genetic variants which impact individuals' response to foods or nutrients (42). Ultimately, the end goal in personalized nutrition is to be able to provide tailored nutritional advice to each specific individual based on phenotypical and genotypical traits as well as lifestyle, environmental and metabolic factors (43). Different technologies are being used to identify factors which may contribute to explaining the inter-individual variation in response to dietary interventions. One such method is metabolomics.

1.7.1 The use of metabolomics in personalized nutrition

Metabolomics refer to the quantitative analysis of small molecules, such as metabolites found in the circulation (44). Global metabolomics aim to quantify all small molecules, while targeted metabolomics focus on smaller sets of metabolites which are often related to each other (44). Metabolomics in the context of personalized nutrition provides knowledge on how foods are metabolized, and more specifically how individuals can metabolize the same type of foods differently. The use of food-derived biomarkers is one way of distinguishing how different individuals metabolize the same type of foods differently, and these biomarkers or metabolites can further provide insights into how foods can impact the health of different individuals (43). Moreover, metabolomics can also be used to group individuals with similar metabolic profiles, referred to as metabotyping or metabolic profiling, which in turn provides opportunities to issue more precise and personalized nutritional advice to groups with similar metabolic traits (43, 45). The use of metabolomics may also provide opportunities in terms of being able to differentiate between responders and non-responders to nutritional interventions.

Applying metabolomics and biomarkers to investigate their ability to predict weight loss response to diets has not been extensively researched to date. However, some studies have

applied targeted metabolomics to identify biomarkers or metabolites which may be used for this purpose (46-49). One study found that fasting baseline levels of acetoacetate, triacylglycerols, phosphatidylcholines, specific amino acids, creatine and creatinine could contribute to explain some of the variation in weight loss success following a low-calorie diet. It was reported that these metabolites potentially could be used to predict weight loss response in morbidly obese individuals (46).

The use of glycaemic markers as predictors of weight loss response to different diets has also been investigated. One study measured baseline fasting plasma glucose and reported that participants with normal glucose tolerance lost more weight following a low-fat, high-carbohydrate diet whereas individuals with reduced glucose tolerance had a more beneficial weight loss response following a high-fat, low-carbohydrate diet (47). Another study investigated whether insulin sensitivity could predict the effectiveness of a low-fat, high-carbohydrate diet and a high-fat, low-carbohydrate diet for weight loss. Similarly to the previous study, it was reported that insulin sensitive individuals lost more weight following a low-fat, high-carbohydrate diet whereas insulin resistant individuals lost more weight following a high-fat, low-carbohydrate diet (48). A third study investigated whether measuring both fasting glucose and insulin at baseline could predict the most effective diet for weight loss in individuals with obesity following either a low-fat, high-carbohydrate diet or a high-fat, low-carbohydrate diet. In this study however, it was reported that among individuals with reduced glucose tolerance, those with high-fasting insulin lost more weight following a low-fat, high-carbohydrate diet whereas those with low-fasting insulin lost more weight following a high-fat, low-carbohydrate diet (49). In light of the two other previous studies using glycemic markers, the findings of this study might thus suggest that there might be additional sub-groups among those with reduced glucose tolerance which may respond differently depending on whether fasting insulin is high or low. In summary, these studies illustrate the potential and possibilities of giving dietary advice based on biomarkers, but also the complexity as there might be sub-groups within the sub-groups who might respond differently. However, it has to be noted that the findings of the first and last-mentioned study using glycemic status as a predictor of weight loss response were based on retrospective statistical analyses of previous randomized controlled studies and participants were not given dietary advice based on their glycemic status.

1.8 One-carbon metabolism and B-vitamin status

The one-carbon metabolism refers to the metabolic reactions where one-carbon units are transferred between different compounds. In one-carbon metabolism, different molecules can act as methyl donors or methyl acceptors, meaning that they either donate or bind a methyl-group. Central metabolic pathways in the one-carbon metabolism are the methionine-homocysteine cycle, the transsulfuration pathway, the folate cycle and the choline oxidation pathway. An overview of these pathways is presented in Figure 1.

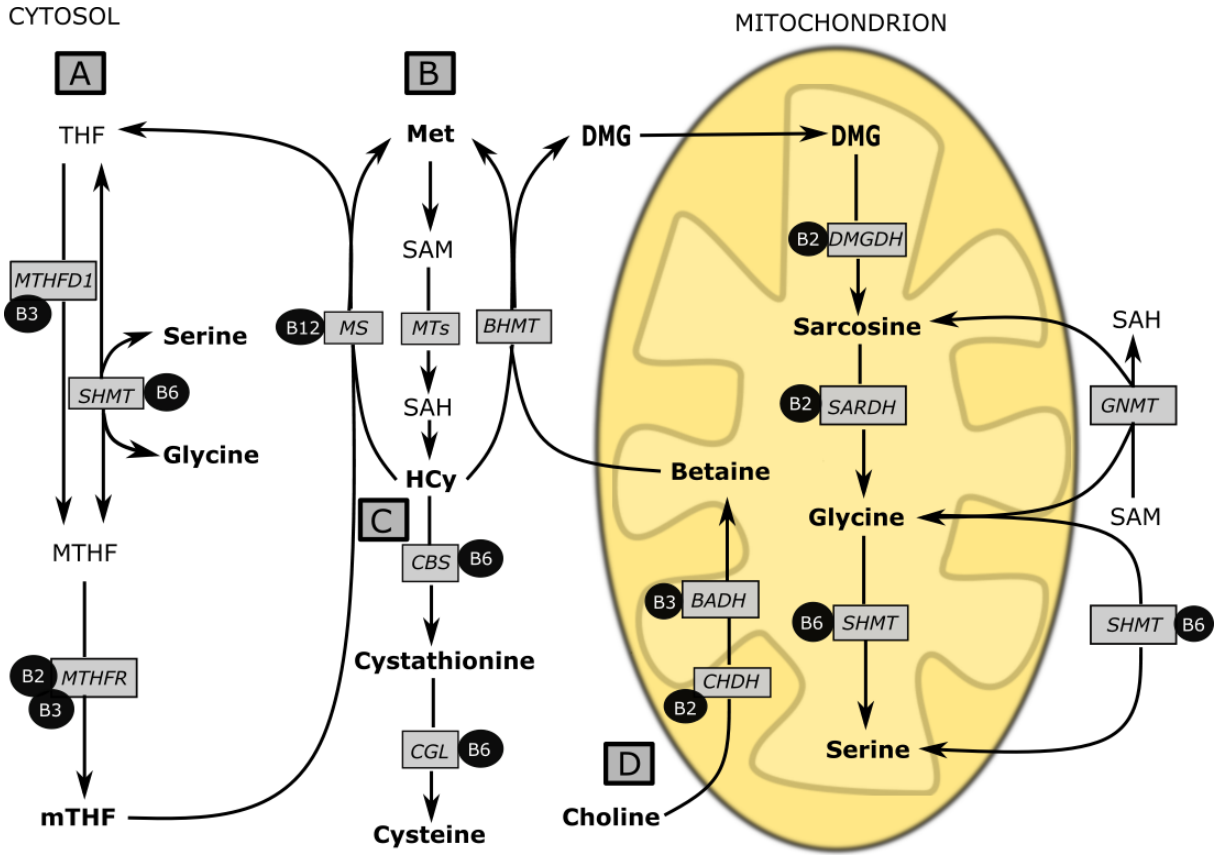

Figure 1. An overview of central metabolic pathways in one-carbon metabolism. A) The folate cycle, B) the methionine-homocysteine cycle, C) the transsulfuration pathway and D) the choline oxidation pathway. The metabolites shown in bold text as well as the B-vitamin cofactors shown in black circles are of relevance to this thesis. The enzymes are presented in grey boxes. THF, tetrahydrofolate; MTHFD1, methylenetetrahydrofolate dehydrogenase complex 1; SHMT, serine hydroxymethyltransferase; MTHF, 5,10-methylenetetrahydrofolate; MTHFR, methylenetetrahydrofolate reductase; mTHF, 5-methyltetrahydrofolate; Met, methionine; SAM, S-adenosylmethionine; MS, methionine synthase; MTs, methyltransferases; BHMT, betaine-homocysteine methyltransferase; SAH, S-adenosylhomocysteine; HCy, homocysteine; CBS, cystathionine-β-synthase; CGL, cystathionine-γ-lyase; CHDH, choline dehydrogenase; BADH, betaine aldehyde dehydrogenase; DMG, dimethylglycine; DMGDH, dimethylglycine dehydrogenase; SARDH, sarcosine dehydrogenase; GNMT, glycine-N-methyltransferase.

1.8.1 The methionine-homocysteine cycle and transsulfuration pathway

A central methyl donor in one-carbon metabolism is S-Adenosylmethionine (SAM) which is derived from the amino acid methionine (50). Whenever SAM donates a methyl group it is converted to S-Adenosylhomocysteine (SAH) which in turn can be converted to homocysteine (51, 52). Homocysteine can further be remethylated back to methionine or go through the transsulfuration pathway to form cysteine. The remethylation of homocysteine back to methionine can happen in two ways and is reliant on the donation of a methyl group provided from metabolites related to either the choline oxidation pathway or the folate cycle. In the folate-dependent remethylation of homocysteine, 5-methyltetrahydrofolate (mTHF) function as the methyl donor in an enzymatic reaction catalyzed by methionine synthase (MS) where vitamin B12 serves as a cofactor (53). The other remethylation pathway uses betaine from the choline oxidation pathway as the methyl donor in a reaction catalyzed by betaine-homocysteine methyltransferase (BHMT) (53). The transsulfuration pathway on the other hand leads to the irreversible removal of homocysteine through two vitamin B6-dependent enzymatic reactions that first leads to the formation of cystathionine and then to the formation of cysteine (54).

1.8.2 The folate cycle

Folate is a collective term that refers to the different coenzyme forms of vitamin B9 (55). Tetrahydrofolate (THF), a reduced form of folate, is the active form of the vitamin and function as a backbone in the folate cycle (55, 56). Naturally occurring folate in food is mainly present in the form of THF and dietary sources include dark green vegetables, liver and beans among others (57). The term folate also includes folic acid, which is a synthetic form of folate used in supplements and fortified foods as it is more stable and has higher bioavailability than natural folate (55, 58). However, folic acid is reduced to THF and further metabolized like the naturally occurring forms of the vitamin (56). Folate is mainly found in the circulation in the form of mTHF (57, 59).

The folate cycle refers to the metabolism of folate inside our cells which provide the different coenzyme forms of folate. The first step in the folate cycle is the conversion of THF to 5,10-methylenetetrahydrofolate (MTHF) which can occur in two ways. The one way is through a three-step reaction catalyzed by the enzyme methylenetetrahydrofolate dehydrogenase complex 1 (MTHFD1) which uses vitamin B3 as a cofactor (60, 61). The other way is

through the enzyme serine hydroxymethyltransferase (SHMT) which utilizes serine as the methyl donor and vitamin B6 as a cofactor leading to the formation of both MTHF and glycine (62). MTHF can further be metabolized with the help of methylenetetrahydrofolate reductase (MTHFR) which utilizes both vitamin B2 and B3 as cofactors to mTHF (63, 64). As mentioned, mTHF function as a methyl donor in the remethylation of homocysteine and this reaction yields THF which once again can go through the folate cycle.

1.8.3 The choline oxidation pathway

The choline oxidation pathway begins with the conversion of choline to betaine inside the mitochondrion (65, 66). This is catalyzed by two enzymatic reactions where the first is dependent on vitamin B2 and the second on vitamin B3 as cofactors (65, 66). Betaine can then diffuse into the cytosol to function as a methyl donor in the remethylation of homocysteine which in addition to methionine also yields dimethylglycine (DMG) (53). DMG can diffuse into the mitochondrion and be further demethylated to sarcosine in a vitamin B2-depedent enzymatic reaction (67). Sarcosine can further be demethylated to form glycine in another vitamin B2-dependent enzymatic reaction (67). From there glycine can either be demethylated to form serine in a vitamin B6-depedent reversible reaction catalyzed by SHMT or be converted back to sarcosine in the cytosol by the help of SAM as a methyl donor in a reaction catalyzed by glycine N-methyltransferase (GNMT) (68, 69).

1.8.4 Markers of B-vitamin status

Riboflavin, B2

Riboflavin or vitamin B2, is a water-soluble vitamin which can be obtained from dietary sources such as meat, dairy and certain vegetables. In plasma, most of the riboflavin is found as free riboflavin whereas most of cellular riboflavin exist as either flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD) which are the active cofactor forms (70, 71). The function of riboflavin is mainly related to serving as cofactors for flavoproteins involved in redox reactions and energy metabolism among others (72). In one-carbon metabolism, riboflavin function as a cofactor for enzymes involved in both the transsulfuration pathway as well as the choline oxidation pathway (73, 74).

Niacin, B3

Niacin is the collective term for nicotinamide (NAM), nicotinic acid (NA) and nicotinamide riboside, commonly referred to as vitamin B3 (75, 76). Niacin can be obtained in the diet through food sources such as meat, vegetables or cereals or through breakdown of the amino acid tryptophan (75). NAM and NA are both precursors of nicotinamide adenine dinucleotide (NAD) whose function is related to serving as a cofactor for enzymatic redox reactions and as an electron carrier in energy metabolism (75). As previously mentioned, several enzymatic reactions related to the folate cycle and choline oxidation pathway also depend on niacin in the form of NAD^+ as cofactor. N1-methylnicotinamide (mNAM) is formed through the breakdown of NAD^+ (77). Assessment of niacin status is often done by measuring the plasma concentration of NAM, NA and mNAM due to the fact that half-life of NAD in plasma is very short (75).

Vitamin B6

Vitamin B6 refers to a total of six compounds that are interconvertible, namely pyridoxal (PL), pyridoxine and pyridoxamine in addition to the phosphorylated forms of these compounds (78, 79). Dietary sources to vitamin B6 are animal sources such as meat and fish, but also whole grains. Vitamin B6 serves as a cofactor for multiple enzymatic reactions and pyridoxal-5-phosphate (PLP) is the active cofactor variant of the vitamin (78, 79). PL, pyridoxine and pyridoxamine can be converted to the active cofactor PLP through the action of different enzymes (78, 79). As mentioned, both the enzymatic reactions in the transsulfuration pathway are dependent on vitamin B6 in the form of PLP as a cofactor in addition to the enzyme SHMT which is responsible for the interconversion of glycine and serine (79). Vitamin B6 exists mostly in the form of PLP in blood, however some of it is also found in the form of PL and 4-pyridoxic acid (PA) (79, 80). Assessment of vitamin B6 status is often done by measuring the concentration of PLP in plasma, however measurement of the total circulating forms of vitamin B6 has been suggested to be more accurate as plasma PLP can be influenced by factors such as smoking or inflammation (79, 80).

Cobalamin, B12

Cobalamin, also known as vitamin B12, is the last of the B-vitamins that will be discussed in this thesis. Dietary sources to cobalamin include meat, fish and dairy as well as many other foods of animal origin (81). In the body, cobalamins function is to serve as a cofactor both for

MS responsible for the remethylation of homocysteine and for metylmalonyl-CoA mutase which catalyzes the breakdown of methylmalonyl-CoA to succinyl-CoA (81, 82). In the case of cobalamin deficiency, both of these enzymes will be inhibited. This in turn will lead to an accumulation of homocysteine in addition to methylmalonic acid (MMA) which is formed in a non-enzymatic reaction from methylmalonyl-CoA (81, 82). Several markers of vitamin B12 exist including total serum cobalamin, total homocysteine and MMA. Nevertheless, none of them have been shown to be superior when utilized separately due to their limitations. For example, in blood most of the cobalamin is bound to haptocorrin and a less amount is bound to holo-transcobalamin (holo-TC) (81-83). The cells however are only able to take up holo-TC and thus measuring total serum cobalamin might not be the best method to assess vitamin B12 status (81, 82). Measurement of total homocysteine as an indicator of vitamin B12 status also have its flaws as homocysteine is dependent on folate and cobalamin to be remethylated (82). Measurement of MMA can be used as an indicator of cobalamin deficiency, although MMA levels may be influenced by factors such as reduced renal function and older age (82, 84).

1.8.5 One-carbon metabolism, overweight and diet

Some of the metabolites discussed have been found to be altered with obesity. Specifically, increased total plasma concentrations of cysteine have been linked to both increased fat mass, obesity and BMI in men and women (85-88). Additionally, lower circulating concentrations of glycine has also been associated with obesity (89). Several studies have also shown that diet influences the circulating concentrations of one-carbon metabolites. For example, one study investigated the effect of a 7-day restriction of the dietary intake of methionine and cysteine while supplementing with polyunsaturated fatty acids (PUFA) and reported reduced plasma concentrations of methionine and cystathionine and increased plasma concentrations of total homocysteine and serine (90, 91). Another study investigated the postprandial effects of a meal low in cysteine and methionine with PUFA-enrichments and reported reduced plasma concentrations of total cysteine (92). Furthermore, in another study, higher concentrations of glycine, serine, cystathionine and riboflavin and lower concentrations of cysteine and cobalamin was observed when replacing approximately 6.5 E% from saturated fatty acids with PUFA (93).

1.9 PPAR-α and personalized nutrition

1.9.1 The relevance of PPARα in relation to nutrition

Peroxisome proliferator-activated receptor α (PPARα) is a nuclear receptor of particular interest in relation to nutrition as it regulates the metabolism of lipids, carbohydrates and amino acids, making PPARα a central factor in energy metabolism (94). Upon activation, either by fatty acids supplied by the diet or produced in the liver in response to a fasting state, PPARα function through upregulating genes that are responsible for β-oxidation. PPARα is consequently profusely expressed in the liver, heart, brown adipose tissue and skeletal muscle which are all tissues with high rates of fat oxidation (95-98). PPARα is not only relevant in the metabolism of lipids, but also has important functions in the metabolism of carbohydrates and amino acids. In cases of sparse glucose availability such as fasting, PPARα will upregulate genes which facilitate gluconeogenesis and consequently downregulate genes that facilitate the storage of glucose in order to ensure stable supply of glucose to the brain and other glucose-dependent tissues (98). Furthermore, PPARα suppress the degradation of amino acids through downregulating the expression of enzymes involved in the catabolism of amino acids (99).

The activation of PPARα can be linked to diet as it supplies fatty acids which act as activators, and especially the long-chained polyunsaturated fatty acids (PUFA's) and related compounds (100). Furthermore, this might imply that the level of dietary activation of PPARα may be influenced by both the amount and composition of fatty acids supplied through the diet (96). Given that both a fasting state and dietary intake of fatty acids activate PPARα, a ketogenic diet is of interest as it consists of a high intake of fats and a low intake of carbohydrates and thereby imitate a fasting state by inducing the use of fats as primary fuel.

1.9.2 Biomarkers of PPARα-activation

Animal studies have shown that activation of PPARα through the administration of PPARα-agonists leads to altered blood concentration of metabolites related to one-carbon metabolism, the choline oxidation pathway and markers of vitamin B status (101, 102). In both of these studies, activation of PPARα lead to marked increases in the plasma concentration of DMG, glycine, serine, NAM, mNAM, PLP, PL and MMA and a reduction in the plasma concentration of riboflavin. Few studies have investigated the effect of PPARα-activation on plasma concentration of metabolites related to one-carbon metabolism in humans, however treatment of humans with fibrates which are PPARα-agonists have been associated with increased plasma concentrations of homocysteine and decreased plasma concentrations of

betaine (103, 104). The findings that the concentration of several metabolites such as those mentioned above are altered with activation of PPARα, suggest that these metabolites potentially can function as biomarkers of PPARα-activity. Furthermore, this may have clinical importance with regards to personalized nutrition advice as PPARα is an important regulator of energy metabolism and specifically increases the metabolism of fat upon activation. Given the important role of PPARα in the regulation of energy metabolism, dietary response may differ in individuals with high or low endogenous PPARα-activity, respectively. This is of particular interest when considering diets with different carbohydrate and fat content.

Hypothesis and aims

When considering weight loss, there is a substantial inter-individual variation in the response to different diets. Identifying biological factors which may predict who will respond to a given diet has the potential to improve our understanding and treatment of overweight and obesity. Endogenous PPARα-activity may be a key factor influencing the response to diets differing in fat content. The aim of this study was therefore to determine whether baseline plasma concentrations of metabolites related to one-carbon metabolism and B-vitamin status, of which some have been suggested as potential biomarkers of PPARα-activity, was associated with weight loss and moreover could predict inter-individual differences in the weight loss response to two diets with very different fat- and carbohydrate content. We further hypothesized that the metabolites suggested to reflect endogenous PPARα-activity would be similarly associated with weight loss response to the diets.

2 Methods

2.1 Study design

This master project is a sub-study based on data obtained from a randomized controlled trial (FATFUNC, ClinicalTrials.gov, Identifier NCT01750021) investigating the effects of two diets differing in fat and carbohydrate content on total body weight, visceral fat mass, abdominal subcutaneous fat mass and waist circumference and other parameters of metabolic importance in obese men (105). The original study was carried out in Bergen, Norway from January to May 2013. The recruitment of participants was done through a post in the local newspaper with a description of the project. The inclusion and exclusion criteria for the study were as follows:

Inclusion criteria:

- Male
- Age between 30-50 years
- Abdominal obesity
- Normal fasting blood glucose < 7 mmol/L
- Waist circumference > 98 cm
- BMI > 29 or percentage body fat ≥ 25

Exclusion criteria:

- Severe diseases including inflammatory bowel diseases or known food allergies
- Regular medication except alkalizing gastric buffers
- Attempts at systematic weight reduction over the previous 6 months
- Regularly consuming > 2 alcohol units per week

Two pre-screenings were performed to secure that all participants met inclusion criteria by the start of the intervention. Six men did not meet inclusion criteria after pre-screening and additionally four recruited participants withdrew before randomisation. During the study period of 12 weeks, the participants came in for study visits at baseline, 4, 8 and 12 weeks.

2.2 Diets

46 participants were randomly allocated to either following a low-fat, high-carbohydrate diet (LFHC) or a very-high fat, low-carbohydrate diet (VHFLC). The LFHC-diet consisted of

30% of total energy intake from fat and 53 % of total energy intake from carbohydrates whereas the VHFLC-diet consisted of 73% of energy from fat and 10% energy from carbohydrates. Both the LFHC- and the VHFLC-diet were similar in terms of protein (17 E %) and energy content (~2090 kcal). Information on their diet group allocation was disclosed to each participant after baseline measurements and samples were taken.

Participants were provided with a recipe booklet consisting of more than hundred pre-specified meals each of which was designed to have the exact macronutrient profile of the respective diets. The recipes included minimal amounts of highly processed foods and added sugar. Participants were also asked to take a vitamin and mineral supplement without iron (Solaray Spektro) for 8 weeks before the start of the intervention to limit individual differences in macronutrient status that might influence energy metabolism. More detailed descriptions of the intervention as well as the composition of the diets have been previously published (105).

2.3 Body weight measurements

Measurements of body weight were performed at each study visit. Body weight measurements of the participants were taken while barefoot and in light clothing by using InBody 720; Biospace which is a segmental multifrequency bioelectrical impedance measurement system. Participants were also asked to use the restroom and to stand in an upright position for over 5 minutes prior to the measurements.

2.4 Blood samples and quantification of metabolites

Blood samples of venous blood including whole blood, plasma and serum was collected from the participants during fasting conditions (overnight or for ≥10 hours) at every study visit. Following preparation, the blood samples were stored at -80°C. For this sub-study, the metabolites of interest were one-carbon metabolites related to the methionine-homocysteine cycle, transsulfuration pathway, choline oxidation pathway and markers of B-vitamin status. Both the analysis of one-carbon metabolites as well as B-vitamins were performed at Bevital A/S, Bergen, Norway (http://bevital.no/), where sample handling was carried out by robotic workstations. Quantification of the metabolites was performed using gas- or liquid chromatography coupled to tandem mass spectrometry (GC-MS/MS, LC-MS/MS) (106-108) or microbiological assays (109). An overview of the metabolites and the type of quantification method are provided in Table 1.

Table 1. Analytical methods and plasma metabolites

Analytical method	Plasma metabolites	Normal range [1]	ICC
GC-MS/MS	Methionine	18-50 µmol/L	0.33
	Homocysteine	<15 µmol/L	0.72
	Cysteine	150-350 µmol/L	0.62
	Glycine	150-300 µmol/L	0.81
	Serine	95-125 µmol/L	0.71
	MMA	<0.26 µmol/L	0.81
	Sarcosine	0.7-2.3 µmol/L	0.68
	Cystathionine	<0.4 µmol/L	0.63
LC-MS/MS	Choline	5-15 µmol/L	0.36
	Betaine	20-60 µmol/L	0.65
	DMG	1.5-5 µmol/L	0.64
	PLP	15-150 nmol/L	0.70
	PL	5-150 nmol/L	0.62
	PA	10-200 nmol/L	0.58
	Riboflavin	5-100 nmol/L	0.79
	FMN	3-30 nmol/L	0.69
	NAM	100-600 nmol/L	NA
	mNAM	20-250 nmol/L	NA
	Folate (mTHF)	>7.5 nmol/L	0.56
Microbiological assay	Cobalamin	>150 pmol/L	0.82

ICC indicates intraclass correlation coefficient; DMG, dimethylglycine; FMN, flavin mononucleotide; NAM, nicotinamide; mNAM, N1-methylnicotinamide; PLP, pyridoxal-5'-phospate; PL, pyridoxal; PA, pyridoxic acid; MMA, methylmalonic acid; NA, not available.

[1] Normal range values retrieved from bevital.no

Metabolite concentrations are not static and varies during both short- and long-term. The intraclass correlation coefficient (ICC) is a descriptive statistic quantifying the between-subject variation relative to the total variation and is frequently reported as a measure of within-person reproducibility. ICC lies within 0-1, where higher values indicate better within-person reproducibility (a higher proportion of the variation is between-subjects) and represent the extent to which a single measurement reflects long-term average exposure within a person. The conventional cutoffs are <0.4 which is considered to be reflect poor reproducibility and values >0.75 is considered to reflect excellent reproducibility. The ICC of

the metabolites included in this thesis are generally reflecting good to excellent reproducibility.

2.5 Statistical analyses and presentation of data

The data was explored according to the diet groups. Baseline characteristics and baseline plasma concentration of metabolites are presented as means (standard deviations; SD) or counts (%) for each group and for the total population. In addition, body weight and BMI at 4, 8 and 12 weeks are also presented as means (SD). The overall association between baseline plasma metabolite concentrations and subsequent weight loss were assessed by calculating spearman correlations with weight change in % at 12 weeks. Inverse correlations indicate that increasing metabolite concentration was associated with larger weight loss. To visualize the association between baseline metabolite concentration and weight loss, weight loss in % from baseline to 12 weeks was plotted against the metabolite concentrations at baseline. The individuals were coloured according to diet group, and linear regression lines extrapolated to the range of the metabolites were superimposed on the plot to visualize trends within the diet groups. To formally test the potential interaction between the baseline metabolite concentrations and the diets, an interaction term was added to a linear regression analysis comparing body weight at week 12, adjusted for baseline body weight. For the purpose of data visualization in order to look for trends, the following extreme data points, outside the normal range, were omitted from the plots: 59.03 for methionine µmol/L, 1.0 µmol/L for cystathionine, 9.88 µmol/L for DMG and 48.29 nmol/L for FMN. All data were however included in the linear regression analyses to formally evaluate the potential interaction. Spearman correlations between all metabolites at baseline, as well as markers of glycaemic status previously used to predict response to diets differing in fat and carbohydrate content, are presented in a correlation matrix.

The statistical analyses were performed with R version 4.0.3 (2020-10-10) for Mac, and the following packages within the tidyverse; ggplot, broom, corrr and rmarkdown. Microsoft Excel version 16.44 (20121301) was also used for organization of the tables. Inkscape was the software used to make vector graphics.

2.6 Ethics statement

The study was approved by the Regional Ethics Committee (2011/2282/REK west) and conducted according to the criteria set by the Declaration of Helsinki. All participants gave

written informed consent. The dataset provided for data analysis were anonymized to secure that no participants could be identified.

3 Results

3.1 Baseline characteristics

Of the 46 participants randomized to follow either the LFHC- or VHFLC-diet, only 38 men were included in the data analysis. Eight participants were excluded, whereof two participants withdrew before baseline visits and four dropped out early during follow-up. Additionally, two participants from the VHFLC-group were excluded due to lacking compliance on diet records and data collected at study visits. The data analysis thus consisted of 18 participants in the LFHC-group and 20 in the VHFLC-group.

Table 2 provides baseline characteristics for the LFHC- and VHFLC-group and for the total population. The mean age was equal in the two groups at 40.2 years. There were twelve smokers in the total population, corresponding to six smokers in the LFHC-group (33.3 %) and six smokers in the VHFLC-group (30.0 %). Both diet groups had a similar reduction in body weight in total and at the different time points from baseline to week 12 where the mean weight loss were 12.2 kg (11.0 %) in the LFHC-group and 12.0 kg (10.5 %) in the VHFLC-group, respectively. Similarly, BMI decreased by 3.7 points in the LFHC-group compared to 3.5 points in the VHFLC-group during the study period.

Baseline characteristics of the metabolites are also presented in Table 2 for both diet groups and for the total study population. In the methionine-homocysteine cycle and transsulfuration pathway, the baseline mean concentration of methionine and cysteine were slightly higher in the LFHC-group whereas the baseline mean concentration of homocysteine and cystathionine were slightly higher in the VHFLC-group. For the metabolites related to the choline oxidation pathway, the baseline mean concentration of choline, betaine, sarcosine, glycine, and serine were all slightly higher in the LFHC-group whereas the baseline mean concentration of DMG were marginally higher in the VHFLC-group. The baseline mean concentration of sarcosine were also slightly above normal range in both diet groups and in the total population.

Table 2. Baseline characteristics of the study population

Variable	Total population	LFHC	VHFLC	r [1]
Age (years)	40.2 (5.00)	40.2 (4.50)	40.2 (5.53)	
Smoking[2]	12 (31.5 %)	6 (33.3 %)	6 (30.0 %)	
Body weight (kg)				
Week 0	112 (11.6)	111 (13.8)	114 (9.47)	
Week 4	106 (11.0)	104 (13.0)	107 (9.03)	
Week 8	103 (10.5)	101 (12.5)	104 (8.36)	
Week 12	100 (9.90)	98.8 (11.8)	102 (7.93)	
BMI (kg/m^2)				
Week 0	33.9 (2.99)	33.6 (3.62)	34.1 (2.35)	
Week 4	31.9 (2.87)	31.5 (3.50)	32.2 (2.21)	
Week 8	31.0 (2.78)	30.7 (3.39)	31.3 (2.13)	
Week 12	30.3 (2.64)	29.9 (3.29)	30.6 (1.90)	
Metabolites				
One-carbon metabolites, µmol/L				
Methionine	28.0 (6.62)	28.7 (4.33)	27.4 (8.23)	- 0.15
Homocysteine	9.89 (1.99)	9.82 (2.09)	9.96 (1.95)	0.08
Cystathionine	0.22 (0.14)	0.20 (0.048)	0.23 (0.20)	0.04
Cysteine	320 (31.8)	326 (36.1)	315 (27.2)	0.06
Choline oxidation pathway metabolites, µmol/L				
Choline	10.1 (1.57)	10.3 (1.76)	9.86 (1.40)	0.17
Betaine	34.3 (6.57)	35.0 (6.45)	33.7 (6.78)	- 0.18
DMG	4.13 (1.23)	4.06 (0.76)	4.20 (1.56)	0.22
Sarcosine	2.81 (0.46)	2.89 (0.45)	2.73 (0.47)	0.11
Glycine	201 (31.3)	211 (28.1)	192 (31.9)	0.10
Serine	105 (16.7)	107 (18.9)	102 (14.7)	- 0.07
Markers of B-vitamin status				
<u>*B2 vitamers, nmol/L*</u>				
Riboflavin	32.6 (20.0)	37.2 (24.8)	28.4 (13.9)	- 0.10
FMN	18.2 (7.78)	19.7 (9.31)	16.9 (6.02)	- 0.20
<u>*B3 vitamers, nmol/L*</u>				
NAM	791 (172)	821 (176)	764 (168)	0.10
mNAM	200 (72.4)	202 (56.6)	198 (85.7)	0.21
<u>*B6 vitamers, nmol/L*</u>				
PLP	109 (56.0)	113 (61.3)	105 (52.2)	0.00
PL	26.1 (19.1)	30.5 (24.1)	22.1 (12.3)	- 0.15
PA	66.5 (55.8)	80.0 (70.1)	54.3 (36.6)	- 0.12
Folate, nmol/L	15.3 (6.75)	15.5 (4.77)	15.1 (8.26)	0.06

Cobalamin, pmol/L	527 (121)	566 (136)	496 (99.4)	0.23
MMA, µmol/L	0.14 (0.049)	0.14 (0.054)	0.14 (0.045)	0.12

All variables are presented as mean (SD) except smoking which is presented as counts (%). DMG indicates dimethylglycine; FMN, flavin mononucleotide; NAM, nicotinamide; mNAM, N1-methylnicotinamide; PLP, pyridoxal-5'-phospate; PL, pyridoxal; PA, pyridoxic acid; MMA, methylmalonic acid; LFHC, low-fat, high-carbohydrate diet; VHFLC, very high-fat, low-carbohydrate diet.

[1] Spearman´s Rank correlation coefficient of the relationship between weight loss and baseline metabolite concentrations. Inverse correlations indicate that increasing metabolite concentration was associated with larger weight loss.

[2]Smoking is calculated from plasma cotinine > 85 nmol/L.

Concerning the B-vitamins, we observed slightly higher baseline mean concentrations of the B2 vitamers riboflavin and FMN in addition to the B3 vitamers NAM and mNAM in the LFHC-group compared to the VHFLC-group (Table 2). The baseline mean concentration of NAM were also slightly above normal range in both diet groups and in the total population (Table 2). The baseline mean concentration of the B6 vitamers PLP, PL and PA were also somewhat higher in the LFHC-group compared to the VHFLC-group (Table 2). No between-group differences in baseline mean concentration of folate and MMA was observed, however baseline mean concentration of cobalamin were slightly higher in the LFHC-group compared to the VHFLC-group (Table 2). Although some between-group differences in the baseline concentration of the different metabolites were observed, the between-group differences were generally small, as expected in a randomized study.

The Spearman's rank correlation coefficient of the association between the different metabolites at baseline and weight change demonstrated that baseline concentrations of homocysteine, cystathionine, cysteine, choline, DMG, sarcosine, glycine, NAM, mNAM, folate, cobalamin and MMA were positively associated with weight change indicating that lower concentrations were associated with more weight loss (Table 2). The opposite was observed for methionine, betaine, serine, riboflavin, FMN, PL and PA where higher concentrations were associated with more weight loss. However, all the correlation coefficients were close to zero and thus no strong associations were observed (Table 2).

Figure 3 shows the Spearman correlation between all the metabolites as well as markers of glycemic status. The markers of glycemic status were glucose, $HbA1_c$, homeostatic model assessment of insulin resistance (HOMA1-IR) and insulin. The metabolites that were most strongly positively correlated with the markers of glycemic status were DMG and cystathionine. The metabolite with the strongest negative correlation with the markers of glycemic status was mNAM. Glycine was also negatively correlated with all the markers of glycemic status with the exception of glucose.

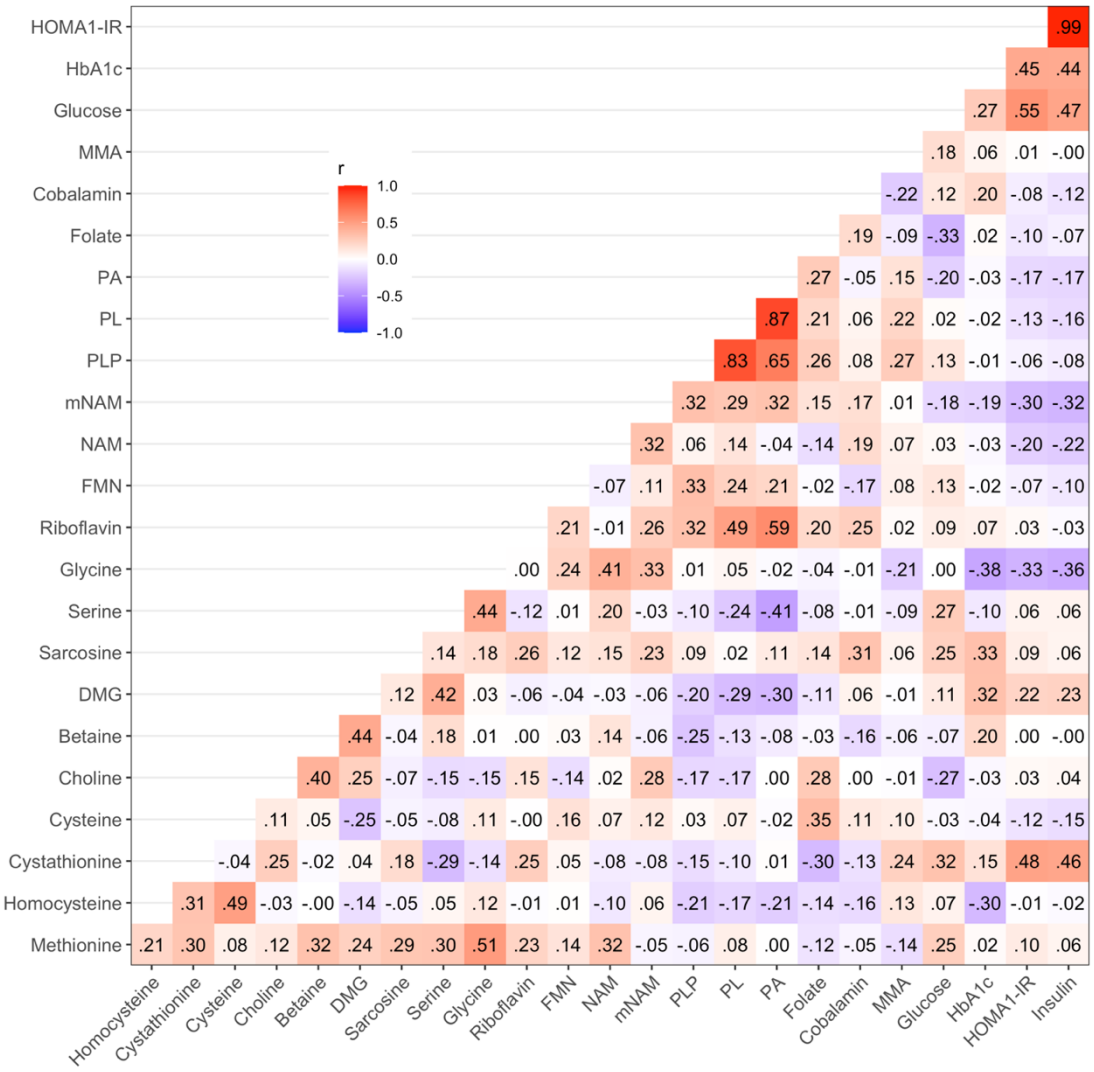

Figure 2. Spearman correlation matrix of the correlation between baseline values of all one-carbon metabolites as well as markers of glycemic status.
Values close to 1 indicate a strong positive relationship; an increase in one variable also leads to an increase in the other variable. Values close to -1 indicate a strong negative relationship as the value of one variable increase the value of the other variable decrease. DMG indicates dimethylglycine; FMN, flavin mononucleotide; NAM, nicotinamide; mNAM, N1-methylnicotinamide; PLP, pyridoxal-5'-phospate; PL, pyridoxal; PA, pyridoxic acid; MMA, methylmalonic acid; HbA1c, haemoglobin A1c; HOMA1-IR, homeostatic model assessment of insulin resistance.

3.2 Plasma concentration of metabolites and weight loss response to diets

3.2.1 Metabolites of the methionine-homocysteine cycle and transsulfuration pathway

Figure 3 illustrates the observed relationship between baseline metabolite concentrations and weight loss response (%) following the LFHC- and VHFLC-diet. For metabolites related to the methionine-homocysteine cycle and transsulfuration pathway the most notable finding was for homocysteine. The LFHC-diet trended towards being most effective for weight loss for lower baseline concentrations of homocysteine whereas the VHFLC-diet trended towards being more effective when homocysteine was higher. For cystathionine, between group differences in weight loss response were small for lower concentrations, however the LFHC-diet appeared to be more effective compared to the VHFLC-diet with increasing concentrations, although this observation appeared to be heavily influenced by a few individual observations. For methionine and cysteine, no specific trends were observed.

3.2.2 Choline oxidation pathway metabolites

Some interesting trends were also observed when analyzing the metabolites in the choline oxidation pathway (Figure 3). For choline, low baseline concentrations were observed to be associated with a larger weight loss compared to higher concentrations irrespective of diet type which was also indicated by the correlation in Table 2. Furthermore, between group differences in weight loss response for different concentrations of choline were small. Between group differences in weight loss response for lower concentrations of betaine were small, but the LFHC-diet trended towards being more effective for higher concentrations of betaine. Regarding DMG, low baseline concentrations were associated with the highest weight loss response irrespective of diet type. Nonetheless, the LFHC-diet were more effective compared to the VHFLC-diet when DMG concentrations were higher. Concerning sarcosine, the LFHC-diet trended to be more effective for lower concentrations whereas the VHFLC-diet trended to be more effective for higher concentrations. The opposite pattern was seen for glycine, where the VHFLC-diet seemed to be more effective for lower concentrations while the LFHC-diet slightly outperformed the VHFLC-diet when glycine concentrations were higher. Regarding serine, higher concentrations tended to be associated with a slightly higher weight loss irrespective of diet type, but not specific differences were observed between the diet groups.

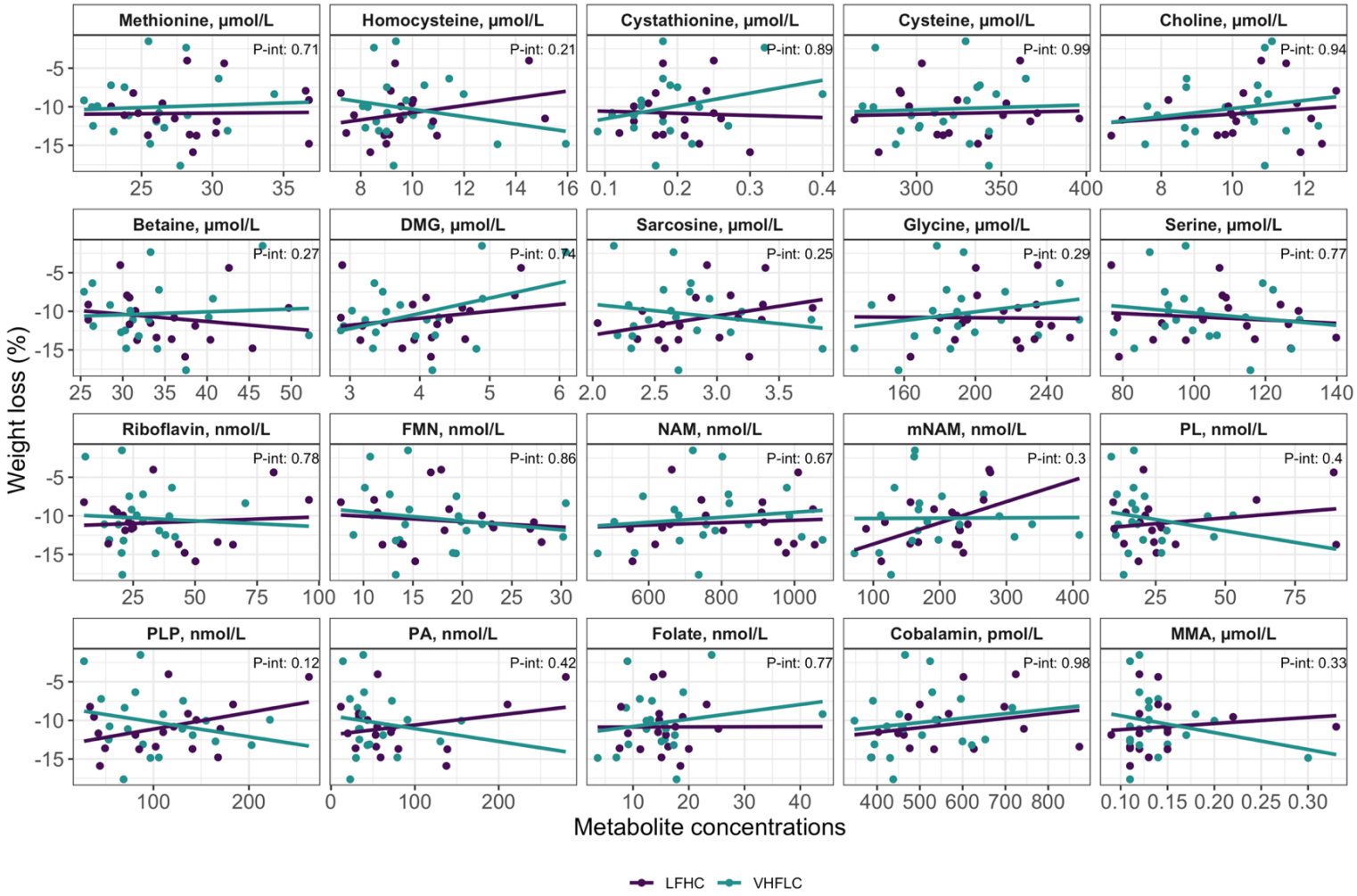

Figure 3. Results on metabolite concentrations and weight loss (%) following the LFHC- and VHFLC-diet.

P-int indicates the P-values of interaction between baseline metabolite concentrations and diet type. DMG indicates dimethylglycine; FMN, flavin mononucleotide; NAM, nicotinamide; mNAM, N1-methylnicotinamide; PL, pyridoxal; PLP, pyridoxal-5'-phospate; PA, pyridoxic acid; MMA, methylmalonic acid.

3.2.3 Markers of B-vitamin status

Some trends were also observed when analyzing the markers of B-vitamin status (Figure 3). The observed trends for the B2 vitamers riboflavin and FMN were modest. For riboflavin, the LFHC-diet tended to be slightly more effective when concentrations were in the lower range, whereas the VHFLC-diet tended to be a bit more effective for higher concentrations, however between-group differences were small. No clear trend was observed between diet type and metabolite concentrations for FMN, but higher concentrations tended to result in a slightly larger weight loss compared to lower concentrations irrespective of diet type. No clear trends were observed for the B3 vitamer NAM. Regarding mNAM, the LFHC-diet trended to be the most effective for weight loss the lower the concentrations. The VHFLC-diet on the other hand, were equally effective regardless of mNAM concentration, but outperformed the LFHC-diet when initial mNAM concentrations were higher. The B6 vitamers PL, PLP and PA all showed similar trends. The LFHC-diet tended to be more effective for weight loss the lower the concentration of these metabolites, whereas the VHFLC-diet were more effective when concentrations were higher, albeit there were few participants with high concentrations of PL and PA.

Concerning folate, the VHFLC-diet showed an equal weight loss response for all baseline concentration values. The LFHC-diet on the other hand trended towards being most effective for lower baseline concentrations and less effective for higher concentrations. No clear trend was observed between diet type and metabolite concentration of cobalamin, but lower concentrations seemed to be associated with a slightly larger weight loss response compared to higher concentrations irrespective of diet type. Concerning MMA, the LFHC-diet tended to be more effective for lower concentrations compared to the VHFLC-diet. The VHFLC-diet however was observed to be more effective the higher the concentrations, however there were few participants with high MMA concentrations.

To summarize, some differences in the response to the diets in terms of weight loss, were observed according to the baseline plasma concentration of the metabolites. Notably, the LFHC-diet outperformed the VHFLC-diet for lower baseline concentrations of homocysteine, sarcosine, mNAM, PL, PLP, PA and MMA and for higher concentrations of cystathionine, betaine, DMG, glycine and folate (Figure 3). The VHFLC-diet produced the superior result when concentrations of glycine were lower and for higher concentrations of homocysteine,

sarcosine, mNAM, PL, PLP, PA and MMA (Figure 3). Despite observing between-group differences in weight loss response for high- and low baseline concentrations of several metabolites, the formal tests for interaction between metabolite concentrations and diet type were inconclusive and indicated that the data did not provide good evidence against the null hypothesis of no interaction.

4 Discussion

4.1 Methodological strengths and limitations

In this study among obese men, we studied whether metabolites related to one-carbon metabolism, the choline oxidation pathway and markers of B-vitamin status were associated with weight loss and whether they could predict inter-individual differences in the weight loss response to a low-fat, high-carbohydrate diet and a very high-fat, low carbohydrate diet. However, the results of the current study should be interpreted in light of some strengths and limitations which will be discussed in the following sections.

4.1.1 Strengths

One strength of the study is the relatively long duration of the dietary intervention. Previous studies have suggested that it may take between three to six weeks for the body to metabolically adapt from utilizing carbohydrates to fat as the primary energy source (110, 111). Considering that the dietary intervention in the current study lasted for 12 weeks, it is less likely that our results may have been influenced by lack of metabolic adaptation to the VHFLC-diet. On the other hand, a 12-week follow-up is insufficient in terms of investigating whether measuring metabolite concentrations can predict the most effective diet for weight loss in the long term, that is beyond 12 weeks.

Adherence to the prescribed diets is generally a challenge in dietary intervention studies. However, in this study the participants were closely monitored, as they were asked to record their dietary intake throughout the study, using an online food record system (105). Food records are considered a reactive dietary instrument, meaning that their use influences behavior, i.e., people change their diet when recording. It is established that people alter their eating behavior when being monitored (112). Therefore, the use of food records likely contributed to increased adherence to the assigned diets as the participants to a greater extent were reminded to follow the diets they were assigned to. To further verify adherence to the diets in the current study, respiratory exchange ratio (RER) was calculated which can be used as an indicator of whether carbohydrates or fat is the primary source of energy supply to the body (105). The RER demonstrated that the intake of fat increased substantially in the VHFLC-group which further supports that the participants adhered to the assigned diets.

Another strength of the study is the randomized controlled design which contributes to minimize potential bias and confounding factors in either one of the diet groups influencing the results as well as making the groups comparable. When done properly, any potential differences between the groups should after randomization thus be due to chance. In the current study we observed that the average baseline concentration of the metabolites as well as mean age and baseline body weight were relatively similar between the two diet groups which points to a successful randomization of the participants. Furthermore, the participants included in the study were relatively homogenous in terms of all being male, middle-aged and having no severe diseases or diabetes which could have impacted the results in terms of increasing the inter-individual variability in response to the diets. On the other hand, studying such a homogenous group of men may reduce the external validity of the study and thus limit our ability to extrapolate our findings outside the study population. Indeed, some argue that one should not necessarily strive for representative samples to improve generalization as population representative samples might reduce internal validity by increasing variability (113). Increased variability in a study sample further makes it more difficult to detect any potential effect of a given intervention.

An additional strength of the study is the fact that the blood samples of the participants were taken in a fasting state, preventing a large influence from recent dietary intake on biomarker concentrations. Further, the activity of PPARα is induced in the fasting state and any individual differences in endogenous PPARα-activity, as measured through the suggested biomarkers, should therefore be better reflected in the fasting state as opposed to if the measurements were taken in the fed state. However, some factors might have contributed to some of the metabolites being less reflective of endogenous PPARα-activity and thus also impacted the usefulness of these as reliable predictors of weight loss response. For example, the participants were asked to take a supplement which contained riboflavin, niacin, pyridoxine, folate and cobalamin (Solaray Spektro) for 8 weeks before the start of the intervention. Considering that we use vitamers of these B-vitamins as biomarkers of endogenous PPARα-activity, the use of this supplement might have led to bias in terms of not being representative of PPARα-activity, but rather a measure of adherence to the use of the supplement. Nevertheless, participants in both groups were asked to take the vitamin- and mineral supplement so it should not contribute to differences between the groups. The second factor which might have impacted the usefulness of the metabolites as predictors of weight loss response to the diets is the inclusion of smokers as smoking has been shown to influence

several metabolites related to one-carbon metabolism and thus may also have influenced the metabolites used in this study as predictors of weight loss response (78, 114). However, as the number of smokers were equal in both diet groups, this is not expected to contribute to between-group differences albeit it does not exclude the possibility of influence due to the potential differences in smoking intensity.

The outcome measure of interest in this study was weight loss. Weight loss can be expressed both in absolute and relative terms. When visualizing the association between baseline metabolite concentration and weight loss in Figure 3, we chose to report weight change from baseline to the end of the intervention in % as opposed to absolute weight change. This can be considered a strength as % weight change often is the preferred outcome measure in clinical settings, but also because absolute weight change is more affected by differences in baseline body weight. Using % weight change can therefore be a way of standardizing this. Further, absolute weight change can also be expected to be larger among those with higher initial body weight, partly due to regression of the mean. Thus, absolute weight change is not directly comparable between individuals unless baseline body weights between the individuals are similar. In the formal tests for interactions between the baseline metabolite concentrations and the diet groups, we compared the body weight at the end of the intervention adjusted for baseline body weight. This is the recommended method when evaluating between-group differences in continuous outcome measures (115).

Some strengths should be mentioned related to the exposure metabolites. Biomarker quantification were performed by trained personnel at Bevital A/S, using established automated methods based on mass spectrometry. In general, the ICC of the metabolites are considered good to excellent, limiting the possibility of misclassification when ranking the participants according to metabolite concentration. Further, we used a targeted metabolomics approach, focusing on the metabolic pathways related to one-carbon metabolism, as well as markers of B-vitamin status, and all results are transparently reported regardless of outcome.

4.1.2 Limitations

Given the exploratory nature of this study, we chose to primarily analyse the data graphically as the main objective was to look for overall trends. However, we did conduct a formal test for the interaction between the diets and the baseline metabolite concentrations. For a long time, an important aspect of scientific research has been null-hypothesis testing where

observations are considered to be statistically significant if the p-value is below a certain threshold which is often set to be p=0.05. Consequently, if the p-value is >0.05, the observations are considered to be statistically insignificant. However, this dichotomous way of deeming observations to be significant or not has garnered much criticism over the past years as it is often misinterpreted and moreover may contribute to wrong interpretations of results (116-118), as well as selective reporting contributing to publication bias, overestimation of effect sizes and the replication crisis (119). Additionally, the p-value does not measure how large or important an observed effect is (117, 118). Essentially, the p-values obtained from the tests for interactions herein represents the likelihood of obtaining the current results if in reality no such interactions exist, and the p-value is thus a continuous measure of the compatibility of our data with the entire model of interaction along with all the background assumptions (120). Importantly, the p-value is also subject to being affected by sample size as its calculation is based on variance. In general, the larger the sample size the less variation and conversely, the smaller the sample size the larger variation (121). Thus, small sample sizes provide poor statistical power to detect an interaction and consequently increases the likelihood of not detecting a real effect which is often referred to as a false negative. In addition, it has been pointed out that much larger samples sizes are needed to estimate an interaction compared to estimating a main effect (121). Considering the small sample size and consequently the low power in this study, it is therefore likely that we were unable to discover any potential real effects.

The small sample size also contributed to a few high observations having a relatively large impact on the trend lines for several of the metabolites in Figure 3. Although an attempt was made to remove some of the most extreme observations outside normal range for cystathionine, DMG, methionine and FMN, there were also generally few individuals with concentrations in the higher range of cystathionine, PL, PA, folate and MMA and most individuals had concentrations in the lower range (Figure 3). This might thus have contributed to false or misleading trends especially for high concentrations of these metabolites. A larger sample size might have been able to reduce the impact of the few high observations and further demonstrated clearer trends.

4.2 Discussion of the results

The primary aim of this study was to determine whether baseline plasma concentrations of metabolites related to one-carbon metabolism and B-vitamin status, of which some have been suggested as potential biomarkers of PPARα-activity, was associated with weight loss and moreover could predict inter-individual differences in the weight loss response to two diets with very different fat- and carbohydrate content. We further hypothesized that the metabolites suggested to reflect endogenous PPARα-activity would be similarly associated with weight loss response to the diets.

We did not observe strong associations between baseline plasma metabolite concentrations and weight loss (Table 2). However, we did observe some differential trends in weight loss response according to baseline plasma metabolite concentrations and diet type (Figure 3). Specifically, we observed that the LFHC-diet outperformed the VHFLC-diet for lower baseline concentrations of homocysteine, sarcosine, mNAM, PL, PLP, PA and MMA and for higher concentrations of cystathionine, betaine, DMG, glycine and folate (Figure 3). The VHFLC-diet on the other hand was observed to produce the superior result when concentrations of glycine were low and for higher concentrations of homocysteine, sarcosine, mNAM, PL, PLP, PA and MMA (Figure 3). However, due to small sample size, the formal tests for interaction between metabolite concentrations and diet type were inconclusive. No specific trends in terms of weight loss response according to diet type were observed for baseline concentrations of methionine, cysteine, choline, serine, riboflavin, FMN, NAM and cobalamin (Figure 3).

4.2.1 PPARα-activity and weight loss response to the diets

Considering the important role of PPARα in energy metabolism, we hypothesized that the metabolites previously suggested to reflect PPARα-activity, as demonstrated in animal studies (101, 102), would be similarly associated with weight loss response to the diets. For higher concentrations of some of the metabolites suggested to reflect PPARα-activity, namely mNAM, PL, PLP, and MMA, we observed that the VHFLC-diet appeared most effective (Figure 3). However, for higher concentrations of DMG and glycine, the opposite was observed (Figure 3). On the other hand, for lower concentrations of mNAM, PL, PLP and MMA we observed that the LFHC-diet trended to be most effective, but for lower concentrations of glycine the VHFLC-diet appeared to be slightly more effective (Figure 3).

No particular pattern was observed for riboflavin, NAM and serine (Figure 3). Previous studies in animals have reported markedly increased plasma concentrations of DMG, glycine, mNAM, PL, PLP and MMA following activation of PPARα. In line with the previous studies with the exception of DMG and glycine, higher PPARα-activity, as reflected by higher concentrations of mNAM, PL, PLP and MMA, might suggest a beneficial effect of following a high-fat diet for weight loss. On the contrary, lower PPARα-activity, as reflected by lower concentrations of the above-mentioned metabolites might suggest that a low-fat diet would lead to larger weight loss. However, the observed trends for the metabolites suggested to reflect PPARα-activity were not consistent as DMG and glycine pointed in the opposite direction.

Not observing consistent trends in terms of which diet would be most effective for metabolites proposed to reflect PPARα-activity could be due to several factors. First of all, the metabolites suggested to reflect PPAR-activity were based on studies in rodents treated with PPARα-agonists and it has not been tested whether these findings extrapolate to humans. Hence, whether these metabolites represent PPARα-activity in humans is uncertain. In addition, both the studies in rodents used PPARα agonists with high affinities for PPARα which may have led to more marked changes in the concentration of the metabolites as opposed to what one might observe naturally such as in our study where no such agonists were used. In addition, several differences in PPARα-activation have been observed between rodents and humans. For example, the human liver contains smaller amounts of PPARα compared to rodent liver and studies have also found evidence that human liver cells are less sensitive to PPARα-activation when compared to mice (122, 123). Furthermore, continuous administration of PPARα-agonists induces liver carcinogenesis in rodents, but the same effects have not been observed in humans which further suggests that the effects of PPARα-activation differs between humans and rodents (124). Altogether, as the metabolites previously suggested to reflect PPARα-activity pointed in somewhat different directions it is difficult to conclude whether endogenous PPARα-activity actually influence weight loss response to diets differing in fat- and carbohydrate content as it could be that these metabolites does not in fact represent PPARα-activity in humans. However, measuring some of these metabolites could nevertheless have potential in terms of identifying who will respond to each of the diets as will be discussed in the following paragraph.

4.2.2 Weight loss response to the diets according to metabolic profile

Despite not observing consistent patterns for the metabolites proposed to reflect PPARα-activity in terms of diet effectiveness, several of the metabolites did however demonstrate differential weight loss responses to the two diets depending on high- or low concentrations. This further makes it possible to suggest a metabolic profile of those who may potentially benefit from following a LFHC-diet and those who may be most responsive to a VHFLC-diet. Our results suggest that the metabolic profile of those who potentially will benefit most from following a VHFLC-diet in terms of weight loss to be characterized by lower concentrations of glycine and higher concentrations of homocysteine, sarcosine, PL, PLP, PA and MMA (Figure 3). On the contrary, the metabolic profile of those who may potentially benefit more from following a LFHC-diet in terms of weight loss was observed to be characterized by lower concentrations of homocysteine, sarcosine, mNAM, PL, PLP, PA and MMA and higher concentrations of cystathionine, betaine and glycine (Figure 3).

Measuring all of these metabolites in order to identify certain metabolic profiles which can be used to stratify individuals to the most effective diet might require too comprehensive analyses. However, there may be a potential combination of some of these metabolites which can be used for this purpose. For example, PL, PLP and MMA demonstrated the same trends in terms of which diet was more effective depending on higher or lower concentrations. Further, these metabolites were also observed to be positively intercorrelated (Figure 2), which may imply that measuring these metabolites in combination can be used as a combined biomarker to stratify individuals to the most effective diet according to their metabolic profile. However, considering the exploratory nature of this study and not least the small sample size, the observed patterns and suggested metabolic profiles should be interpreted with caution as it is not possible to rule out random variation as an explanation.

4.2.3 Comparison of the observed trends with studies using glycemic markers

To investigate whether the trends we observed pointed in the same direction as previous studies using markers of glycaemic status to predict weight loss response to diets differing in carbohydrate- and fat-content, we calculated spearman correlations between all metabolites at baseline as well as markers of glycaemic status (Figure 2). The markers of glycaemic status were glucose, $HbA1_c$, HOMA1-IR and insulin. High concentrations of these markers indicate a reduced glucose tolerance and insulin sensitivity whereas lower concentrations indicate a better glucose tolerance and insulin sensitivity. As mentioned, previous studies have reported

a low-fat, high-carbohydrate diet to be most effective for weight loss among obese individuals with normal glucose tolerance and insulin sensitivity and a high-fat, low-carbohydrate diet to be the most effective diet for obese individuals with a reduced glucose tolerance and insulin resistance (47, 48), although a third study suggested that there might be additional sub-groups among those with reduced glucose tolerance which might respond differently depending on whether fasting insulin concentrations are high or low (49). However, in terms of previous findings, we would expect the metabolites that were positively correlated with markers of glycaemic status to trend towards a greater weight loss following the VHFLC-diet when concentrations were high and conversely the LFHC-diet to trend towards being most effective when concentrations were in the lower range. On the contrary, we would expect the metabolites that were negatively correlated with markers of glycaemic status to trend towards a greater weight loss following the LFHC-diet when concentrations were high and the VHFLC-diet to trend towards being most effective when concentrations were lower.

In the current study, the metabolites that were observed to be most strongly correlated with markers of glycaemic status were DMG, cystathionine, mNAM and glycine (Figure 2). DMG and cystathionine were both positively correlated with all markers of glycaemic status and trended towards a greater weight loss following the LFHC-diet when concentrations were high (Figure 2 and 3). Furthermore, mNAM was negatively correlated with all markers of glycaemic status and the VHFLC-diet trended towards being most effective for weight loss when concentrations were high and conversely the LFHC-diet to be most effective when concentrations were lower (Figure 2 and 3). Glycine was also negatively correlated with all markers of glycaemic status except glucose, and high concentrations trended towards a slightly greater weight loss following the LFHC-diet whereas low concentrations demonstrated a marginal greater weight loss following the VHFLC-diet (Figure 2 and 3).

In summary, most of the trends we observed did not point in the same direction as previous studies using markers of glycaemic status to predict the weight loss response to diets with different fat- and carbohydrate content. In fact, it was only glycine that pointed in the same direction as previous studies (47, 48). Nevertheless, lower plasma glycine levels have been observed in individuals with insulin resistance and diabetes mellitus type 2, and improvement of insulin resistance have been associated with increases in plasma glycine concentrations which further supports the observed correlation between glycine and markers of glycaemic status in this study (125). Interestingly, although the observed trends for DMG and

cystathionine were inconsistent with our assumptions based on two of the studies using glycaemic status (47, 48), the observed trends were however in line with the findings of Hjorth et al. (49) which reported that individuals with reduced glucose tolerance and high insulin lost more weight following a LFHC-diet. This further supports the implications of additional sub-groups responding differently among those with reduced glucose tolerance depending on high- or low fasting insulin concentrations.

It is important to note that the extreme outliers that were removed from the data visualization plot were included in the correlation analysis which might have impacted the observed correlations. However, we used the Spearman rank correlation instead of the Pearson correlation which is based on ranks rather than the actual values and thus less sensitive to the impact of extreme values, although one cannot rule out that this might have affected our results. Lack of observing similar trends between the studies might also be due to other factors. For example, the studies using glycemic markers included both genders and differed in follow-up time compared to this study. In addition, although all the studies used glycemic markers as predictors of weight loss response to a high-fat vs. low-fat diet, the dietary composition in terms of the amount of fat, carbohydrates and protein differed compared to our study which might also have had an effect on the results.

4.2.4 Potential of personalized nutrition

The large inter-individual variation in weight loss response to dietary interventions counteract the belief that one diet fits all and further support the concept and need for more personalized nutritional strategies to achieve and maintain weight loss. As mentioned, the ultimate goal of personalized nutrition is to issue unique dietary advice to each individual on the basis of information on dietary intake and habits, physical activity, genetics, metabolomics and microbiota among others. However, such an in-depth and personalized approach may not be realistic nor feasible in a public health perspective as it requires high-cost and very comprehensive analyses to provide tailored advice to a single individual. In addition, considering the high number of people suffering from overweight and obesity in the world such a personalized nutrition approach would have to be implemented at a large scale to have any effect in the broader picture. Moreover, it might actually contribute to increased health disparities by benefitting those who can afford such personalized treatment.

A more feasible and less expensive personalized nutrition approach which may benefit a larger proportion of the population may be to tailor dietary advice to stratified groups with similar characteristics and traits beyond age and gender. Considering the many technologies available to study the relationship between biological characteristics and the response to diets, it might be possible to identify easily measurable factors or characteristics able to identify such subgroups. These characteristics may be used to discriminate between those who will respond to a certain diet and those who will have more benefit by following another type of diet. In the current study we studied a set of metabolites at baseline to investigate whether measuring any of these might be used to predict the inter-individual differences in weight loss response to a LFHC- or a VHFLC-diet. Although we did not observe strong evidence for interactions between our candidate biomarkers and response to the diets, some interesting trends were observed, which should be further explored in larger data sets. Notably, our approach was targeted to specific metabolic pathways, and hence we only included a limited number of metabolites. Expanding the analyses to include other metabolic pathways could have led to the identification of other metabolites able to predict the most effective diet for weight loss. Nevertheless, future similar studies using targeted metabolomics might be able to replicate our findings as well as identify other metabolites able to predict weight loss response to different diets. As mentioned, if several metabolites point towards a beneficial effect of following a certain diet over another then it might be possible to construct a combined biomarker able to stratify individuals to the most effective diet according to their respective metabolic profile.

4.2.5 Adherence

One common assumption in personalized nutrition is that the more we are able to measure in terms of factors which may cause us to respond differently to dietary interventions, the more effective and better the outcome following the personalized dietary advice will be. However, personalized or not, in order for a certain diet or nutritional recommendation to be effective for weight loss it has to lead to a negative energy balance and the individuals receiving the advice actually has to adhere to the diet or recommendations they receive both in the short and in the long term. As mentioned, lack of adherence to assigned weight loss diets, especially in the long-term, can be a problem for individuals trying to lose weight and not least maintain the weight loss. Essentially, the challenge with diets in terms of achieving and maintaining weight loss is getting individuals to actually make lasting changes in dietary behaviour, and the question is whether personalized nutrition advice to a greater extent

improve adherence and contribute to lasting changes in dietary habits compared to general nutrition advice. It has been shown that informing individuals of having an increased genetic susceptibility to develop obesity does increase their readiness to control their body weight (126). However, this alone was not sufficient to alter dietary behaviour, potentially because it may give the impression that overweight or obesity is genetically determined, and hence less controllable as opposed to if it was solely a result of environmental factors. Some studies have investigated whether receiving personalized nutrition advice is more effective than standard dietary advice in terms of changing dietary habits. Interestingly, one study found that the participants were more likely to change their dietary behaviour when receiving the personalized nutrition advice compared to general nutrition recommendations, but the addition of phenotypic or genotypic information did not contribute to further changes in dietary behaviour (127). Although this study did not investigate the effectiveness of personalized nutrition advice for changing dietary habits beyond six months, which is important in terms of achieving long term weight loss and maintenance, it does provide proof-of-concept that receiving personalized dietary advice may improve adherence.

However, it is important to note that although one might be able to identify certain factors which can predict which diet an individual in theory will be most responsive to, it does not necessarily mean that this diet will be the most effective in practice. Food preferences and other contextual factors such as the family and social situation must always be considered. For example, an individual who in theory will respond best on a high-carbohydrate diet might not prefer or like high-carbohydrate foods such as grains or legumes or vice versa, which further may make it difficult to implement and adhere to the recommended diet. Moreover, it might also be difficult to implement the necessary dietary changes in family or social settings. Therefore, it should be emphasized that weight loss can be achieved via different dietary approaches, and that long-term adherence is the key to successful weight loss and maintenance regardless of which approach is chosen (128).

5 Conclusions and future perspectives

In this study, we did not observe strong associations between weight loss and baseline plasma concentrations of metabolites related to one-carbon metabolism and markers of B-vitamin status. However, we did observe differential trends in weight loss response according to diet type and baseline plasma concentration of several of the metabolites. The findings indicate that the VHFLC-diet produce larger weight loss when concentrations of glycine are lower and concentrations of homocysteine, sarcosine, PL, PLP, PA and MMA are higher, while the LFHC-diet was superior when concentrations of homocysteine, sarcosine, mNAM, PL, PLP, PA and MMA were lower and the concentrations of cystathionine, betaine and glycine were higher. Of the metabolites previously suggested to reflect PPARα-activity, we observed that mNAM, PL, PLP and MMA demonstrated similar trends in terms of which diet was more effective according to higher or lower concentrations, but glycine and DMG demonstrated opposite trends. To further explore whether any of these metabolites can be used to tailor dietary weight loss advice to the individual, a first step would be to perform similar studies in other existing dietary intervention trials. Future studies with larger sample sizes could provide important insights as to which of the metabolites, if any, could be used to differentiate between individuals who will respond to either one of the diets. In addition, if a certain set of metabolites exhibit similar patterns, these might have the potential to be used as a combined biomarker able to stratify individuals to the most effective diet according to their respective metabolic profile.

Whether or not the personalized nutrition approach based on metabolic profiling will revolutionize the management of overweight and obesity and contribute to long-term sustained weight loss is still too early to tell. To date, most of the evidence for a beneficial effect of personalized nutrition is based on retrospective or observational studies where reproducibility is low (129). In addition, although knowledge on the factors responsible for the often-observed inter-individual variation to diets or other dietary interventions is increasing there is still a lot that is currently unknown and not least about the interaction between these factors. To confirm whether analyses of metabolites, genetics or other factors can predict weight loss response to diets and moreover that personalized nutrition advice based on such measurements are more effective in changing dietary behaviour than general nutrition recommendations, it is of importance that the most promising factors are tested prospectively, preferably in randomized controlled trials.

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