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**THE INTERACTION BETWEEN
INFLAMMATORY PROPERTIES OF DIET AND
GENETIC VARIATION IN
BODY WEIGHT REGULATION**

Harry Freitag Luglio Muhammad

PENERBIT ANDI

THE INTERACTION BETWEEN INFLAMMATORY PROPERTIES OF DIET AND GENETIC VARIATION IN BODY WEIGHT REGULATION

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DISSERTATION

To obtain the degree of Doctor at the Maastricht University, on the authority of the Rector Magnificus, Prof. dr. Rianne M. Letschert, in accordance with the decision of the Board of Deans, to be defended in Public on Thursday 9 December 2021, at 10.00 hours.

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This thesis is dedicated to all disadvantaged kids with a dream to make a difference in the world.



THE INTERACTION

BETWEEN INFLAMMATORY
PROPERTIES OF DIET AND
GENETIC VARIATION IN

BODY WEIGHT REGULATION

ABSTRACT

Inflammation might play a part in body weight regulation. Previous studies showed that circulating inflammatory markers, inflammatory properties of the diet, and genetic variation in inflammatory markers are associated with obesity. To date, it is not known whether the interaction between inflammation and obesity could also be evaluated in the context of weight change during and after a weight loss program and whether diet might influence this association.

The general objective of this thesis was to evaluate the influence of diet and inflammatory gene variations on inflammation and body weight changes in obesity. The objective was specified into 4 aims: 1) To evaluate the association of the inflammatory properties of the diet with weight regain after a weight loss program as well as with the risk for obesity in a population-based study; 2) To develop a new low calorie dietary regime with low inflammatory property (LCID) for weight loss and evaluate the association between the inflammatory properties of the diet and weight regain; 3) To examine the influence of inflammatory gene variations on the association between obesity and inflammation; 4) To evaluate the interaction between inflammatory gene variations and diet composition and its effect on weight regain after weight loss.

We showed that the inflammatory property of the diet is associated with adipose tissue inflammation, marked by leptin concentration. In a weight loss program, we reported that the inflammatory property of the diet partially explains the variation in weight regain following weight loss. The modification of a standard low-calorie diet (LCD) for weight loss to lower its inflammatory properties (LCID), reduces hs-CRP compared to the LCD, but does not influence changes TNF-alpha, IL-6, or weight. However, the LCID had a positive impact on improving genomic health by elongation of relative telomere length. We showed that variations in the genes for inflammation markers such as *CRP*, *TNFA*, and *IL6* influenced the correlation between obesity and inflammation. Variations in these genes also influenced the correlation between diet composition and weight regain following weight loss. All in all, these data support the hypothesis that inflammation plays a role in the regulation of body weight. This effect is either direct or indirect through interaction with dietary intake.



THE INTERACTION

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CHAPTER 1

General Introduction and Outline of the Thesis



INTRODUCTION

Obesity affects millions of adults and it not only occurs in developed nations but also in developing nations [1]. It is responsible for an increasing economic and health burden due to its contribution to the increased risk of non-communicable diseases [1,2]. Obesity is the result of a long-term or intermittent positive energy balance. This positive energy balance is usually due to the combination of a high energy intake and low physical activity [3]. A combination of a low-calorie diet and exercise is beneficial to induce weight loss among individuals with overweight and obesity [4]. However, studies also show that after weight loss through traditional lifestyle modification, which includes a low calorie diet, weight regain frequently occurs [5-8]. Thus, investigation of factors that influence weight gain following a successful weight loss is needed (Figure 1.1).

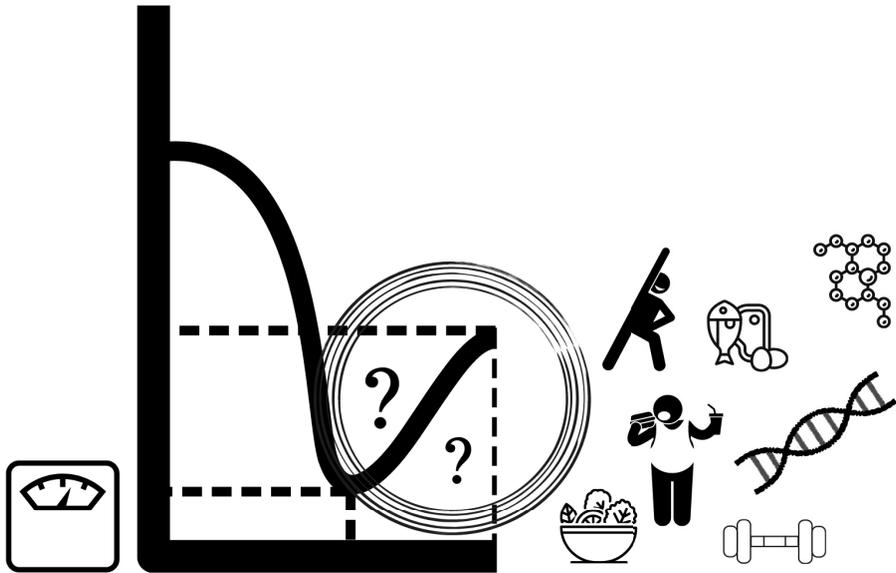


Figure 1.1. Weight regain commonly occurs following weight loss. Several factors were reported to influence this phenomenon, including genetics, hormones, diet pattern, and other lifestyle factors.

Weight loss in individuals with obesity is associated with an increment in adipose tissue stress and inflammation [9,10]. Interestingly, the change of expression of genes associated with adipose tissue inflammation in the first weeks after weight loss is significantly correlated with weight regain after weight loss [9,10]. These findings are also supported by another study showing that inflammatory markers

before weight loss can predict individual resistance towards a weight loss program as well as weight regain following weight loss [11]. Because previous reports have shown the potential role of inflammation in regulating body weight, reducing inflammation might be an important component of a sustainable weight loss program.

INFLAMMATION AND OBESITY: ONE COIN WITH 2 SIDES

Obesity has been associated with increased systemic and adipose tissue inflammation. The excess of adipose tissue is associated with immune cell infiltration and higher production of pro-inflammatory cytokines [12]. In addition, there is a remarkable reduction in anti-inflammatory immune cells in adipose tissue of individuals with obesity. This combination leads to an increment of the inflammatory state of the adipose tissue as well as in the circulation [12]. Studies from a broad range of populations have reported that systemic pro-inflammatory markers, such as high sensitive C-reactive protein (hs-CRP), are higher among individuals with obesity [13]. In addition, several reports suggested that weight loss is associated with a reduction in hs-CRP [14-16].

Interestingly, findings from recent decades indicated that inflammation might also induce obesity and weight gain. First, in a cohort study conducted among 5,062 adult women living in Italy, it was shown that hs-CRP was a predictor of weight gain. Women with a hs-CRP concentration above the median value had a higher risk to increase body weight >14 kg within 20 years [17]. Second, observational studies across different populations showed that variations in pro-inflammatory genes such as *TNFA*, *CRP*, and *IL6* were associated with the risk of obesity [18-20]. Lastly, in a weight loss program, higher baseline inflammation markers were associated with weight loss resistance and higher risk of weight regain [11].

DIET, INFLAMMATION, AND OBESITY

Inflammation is a biological process which involves a variety of cells including immune cells and internal organs. Inflammation plays a crucial part in the host defense system against pathogens [21]. In the last few decades, it has become clear that inflammation is also an important component of the physiological processes, including the body repair system [22] as well as inter-organ communication [23]. Recent scientific developments suggest that inflammation is involved in the pathophysiological process of non-communicable diseases such as atherosclerosis, dyslipidemia, and insulin resistance [24]. In addition, inflammation is also shown to influence genomic health by reducing telomere length [25] and mitochondrial DNA copy number [26]. These alterations are important links between inflammation and the development of chronic diseases [24].

Inflammation is influenced by environmental (e.g., lifestyle, diet, physical activity) factors [27]. It has been reported that the dietary pattern affects circulating inflammatory markers in adults [28]. There are several tools to assess the inflammatory properties of the diet, such as the dietary inflammatory index (DII) [29], the dietary inflammation score [30], and the food-based index of dietary inflammatory potential [31]. Population-based studies in recent years have reported that those scores are associated with systemic inflammation [30-33]. A recent meta-analysis showed that a higher DII was associated with increased body mass index and risk for obesity [34], suggesting that dietary patterns which increase inflammatory markers, could also increase the risk for obesity (Figure 1.2).

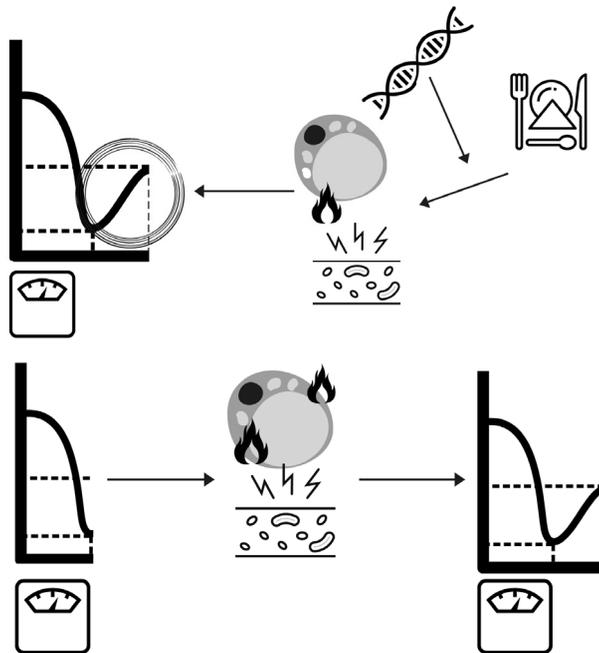


Figure 1.2. Theoretical framework of the interaction between diet and genes on inflammation and its impact on weight regain. During a weight loss program, adipose tissue shrinks and releases inflammatory and stress signals locally and into the blood (below). This is one of the factors that may lead to weight regain after weight loss. There are several factors that influence the adipose tissue inflammation response to weight loss, including diet and genetics (above). Those factors might interact, leading to variation in weight changes following the weight-loss period.

In the calculation of DII, the intake of nutrient and non-nutrient components of the diet are calculated and compiled into an index. Components with pro-inflammatory activity (such as energy, saturated fat, and trans fat) will add to the score, while components with anti-inflammatory properties (such as vitamin C, magnesium, and folate) will reduce the score. After those components are summarized, individuals with the higher score tend to have more circulating inflammatory markers while individuals with a lower score tend to have less [29].

Increased inflammation might play a role in the disruption of several molecular signatures such as mitochondrial function and telomere length. Increased inflammation is associated with the reduction of mitochondrial DNA copy number (mtDNA-CN), an indicator of mitochondrial biogenesis and function [35]. In addition, increased inflammation is associated with more extensive shortening of telomere length [36,37]. Interestingly, it also has been shown that mtDNA-CN and RTL are related to weight gain [38,39]. Thus, it might be argued that mtDNA-CN and RTL are influenced by systemic inflammation due to increased adiposity.

GENES, INFLAMMATION, AND OBESITY

In addition to the environment, inflammation can also be influenced by genetic factors. Population-based studies revealed that certain variations in pro-inflammatory genes are associated with increased inflammation. Gene variations in *TNFA*, *IL6*, and *CRP* have been associated with interindividual differences in proneness to inflammation due to higher circulatory concentration of TNF-alpha, IL-6, and hs-CRP, respectively [40-42]. Furthermore, those gene variations were also reported to be associated with increased risk of obesity [18-20]. This suggests that the risk of obesity can be modulated by variations in genes involved in the inflammation process.

THE INTERACTION BETWEEN GENETIC VARIATION AND DIET AND OBESITY

The mechanism underlying how gene variations in inflammation genes influence the risk of obesity is not well understood. Joffe et al. [43] suggested that dietary intake and variations in *TNFA* and *IL6* genes interact to influence obesity and its comorbidities. For example, among those with the A allele of the *TNFA* -308 G > A gene variation (rs1800629), higher dietary fat intake was associated with a greater risk for obesity compared to those without the A allele (GG). This might explain differences in the individual capacities to increase or reduce inflammation due to a specific dietary regime because of their genetic make-up.

GAP OF KNOWLEDGE

It has previously been shown that the inflammatory properties of the diet are associated with risk of obesity [34]. However, it is not known whether this also applies to the context of weight change during and after a weight loss program. Further, studies also reported that gene variations in inflammation markers are associated with obesity [19,20]. Nevertheless, it is not clear if and how inflammation could regulate body weight and whether the gene variations have an impact on the interaction between diet and inflammation. The studies in this thesis tried to expand knowledge on some of these issues.

AIMS AND OUTLINE OF THE THESIS

Weight regain following weight loss is a major concern in obesity treatment, thus a better dietary approach to prevent weight regain is warranted. It has previously been shown that inflammation plays a role in the development of obesity as well as in the individual response to a weight loss program. Since diet is an important factor that affects systemic inflammation, it seems obvious to evaluate the association between the inflammatory property of diet and weight changes during and after a weight loss program or with adiposity in a population-based study. It might be important to develop a weight loss program with the aim to not only reduce calorie intake but also inflammation. As gene variations in inflammation markers play a role in the development of obesity, studying the potential interaction between gene variations (*TNFA*, *IL6*, and *CRP*) and diet may help to explain the interindividual variations in body weight loss and weight regain.

The general objective of this study was to evaluate the influence of diet and gene variations on inflammation and body weight. The objective is specified into 4 aims: 1) To evaluate the association between the inflammatory property of diet and weight regain after a weight loss program as well as the risk for obesity in a population-based study; 2) To develop a new dietary regime with low calorie - low inflammatory properties for weight loss and evaluate its impact on weight loss and regain; 3) To examine the influence of gene variations on the correlation between obesity and inflammation; 4) To evaluate the relation between gene-diet interaction and weight regain after weight loss.

In Chapter 2, we provide insight in how inflammatory properties of the diet (DII) could affect adiposity measures and leptin, a marker for adipose tissue inflammation in a cross-sectional study among Indonesian adults. A further investigation was done to evaluate whether the inflammatory properties of the diet affected weight changes following a weight loss program in participants of the Dutch YoYo study (Chapter 3).

In Chapter 4, we studied a new dietary regime for a weight loss program called low calorie low inflammatory diet (LCID) in Indonesian adults with overweight or obesity. The aim of LCID was not only to induce a negative energy balance but

also to reduce inflammation during a weight loss program. We further investigated the effect of LCID in Chapter 5. In this chapter, we evaluated the effect of the new dietary regime on genome health as marked by relative telomere length (RTL) and mitochondrial DNA copy number (mt-DNA CN) in the weight loss study in Indonesian adults with overweight or obesity.

In Chapter 6 we studied the influence of variations in inflammatory genes (*TNFA*, *IL6*, and *CRP*) on systemic inflammation, marked by hs-CRP, and on adiposity measures in a cross-sectional study among adults living in North-East England. To confirm findings reported in Chapter 6, an additional analysis with similar genotypes was done to evaluate the influence of variations in inflammation genes (*TNFA*, *IL6*, and *CRP*) on the connection between diet and weight changes after a weight loss program in the DiOGenes study (Chapter 7). The DiOGenes study was a large European trial among individuals with obesity. Participants first lost weight with an energy-restricted diet, and were then randomised to different weight maintenance diets.

The thesis concludes with Chapter 8, in which we provide a summary of all of our findings and discuss suggestions for future research as well as its societal impact.

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CHAPTER 1

General Introduction and Outline of the Thesis

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CHAPTER 2

Dietary Inflammatory Index Score and Its Association with Body Weight, Blood Pressure, Lipid Profile, and Leptin in Indonesian Adults

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ABSTRACT

It was previously reported that dietary intake is an important trigger for systemic inflammation and one of the lifestyle factors for the development of cardiovascular diseases. The aim of this study was to evaluate the association between Dietary Inflammatory Index (DII) score and body weight, blood pressure, lipid profile and leptin in an Indonesian population. This was a cross-sectional study conducted in 503 Indonesian adults. The DII score was calculated based on data of 30 nutrients and food components. Anthropometric profile, blood pressure, lipid profile, and leptin were measured. The association of these variables with the DII score was analyzed. The DII score was not associated with body weight, body mass index (BMI), body fat, waist circumference, hip circumference, systolic and diastolic blood pressure, triglycerides, and high-density lipoprotein (HDL) (both unadjusted and after adjustment for covariates). However, plasma leptin concentration was significantly associated with the DII score ($B = 0.096$, $p = 0.020$). Plasma leptin also increased significantly across tertiles of the DII score (ANCOVA, $p = 0.031$). This positive association between the DII score and plasma leptin concentration suggests a role for the inflammatory properties of the diet in regulating adipose tissue inflammation.

Keywords: dietary inflammatory index; obesity; triglyceride; HDL; leptin

INTRODUCTION

Obesity affects millions of adults and children and this state of over-nutrition is responsible for an increasing economic and health burden worldwide [1,2]. Adiposity is associated with increased risk for non-communicable diseases (NCDs) such as type 2 diabetes mellitus, dyslipidemia, heart disease, and hypertension [1]. One of the mechanisms which may explain the interaction between obesity and the development of NCDs is systemic and adipose tissue inflammation [3,4]. The excess of adipose tissue induces increments in the production of leptin and pro-inflammatory cytokines as well as immune cell infiltration [4]. In addition, a reduction in anti-inflammatory immune cells in adipose tissue of individuals with obesity has been reported [5,6]. This combination leads to an inflammatory state in adipose tissue as well as the circulation of individuals with obesity [4].

Inflammation is also influenced by environmental factors (e.g., lifestyle, diet, physical activity) and genetic background [7]. It has been reported previously that the dietary pattern has an important role in affecting circulating inflammatory markers in adults [8]. Recently, a new tool has been developed to assess the inflammatory properties of the diet: the Dietary Inflammatory Index (DII) [9]. In this index, intake of nutrient and non-nutrient components of the diet are calculated and compiled into an index. The score on this index has been shown to be associated with systemic inflammation [10–12]. In the cross-sectional Spanish PREDIMED (Prevención con Dieta Mediterránea) study the DII score was associated with body weight and other anthropometric measures [13]. In the Spanish SUN (Seguimiento Universidad de Navarra) cohort, the DII score was associated with annual weight gain [14]. We have shown that the DII score was associated with weight regain following a weight loss program in Dutch overweight and obese individuals [15].

Inflammation is associated with the development of chronic diseases and is an important link between obesity and cardiovascular diseases [3,16]. It was previously suggested that inflammation plays an important role in the disturbance of blood pressure and lipid profile [3,16,17]. This notion is supported by several studies in western societies which showed that the inflammatory properties of the diet were associated with early predictors of cardiovascular diseases such as hypertension, and higher plasma triglyceride and lower high-density lipoprotein (HDL) cholesterol concentrations [18–20].

Currently, there is limited evidence on the application of the DII in Asian countries. To our knowledge, there is only one report from a small cross-sectional study conducted in female school teachers in Myanmar, which found no association between the DII score and overweight [21]. Evaluation of the DII in different population settings is important because dietary practices vary amongst cultures and

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might have an impact on the DII value. Additionally, because other factors such as environments, lifestyle, and genetic background are also different among population settings, this might influence the relationship between the DII score and metabolic health. Therefore, the aim of this study was to evaluate the associations between the DII score and body weight, blood pressure, lipid profile, and leptin in an Indonesian population.

MATERIALS AND METHODS

These analyses are part of an Indonesian cohort study that investigates the effect of lifestyle and genetic variation on metabolic syndrome in adults. It is a secondary cross-sectional analysis of baseline data of this study that was carried out among adult men and women between 19 and 56 years of age, living in the urban area of Yogyakarta, Indonesia. A total of 503 individuals, stratified for sex, were recruited from randomly selected neighborhoods from five sub-districts which were selected

The inclusion criteria were: permanent residence (at least 2 years) in the area and agreement to become subject of this study by signing the informed consent. The exclusion criteria were: diagnosis of chronic diseases such as diabetes, cardiovascular disease, or cancer, pregnancy at the time the study was conducted, current or prior cigarette smoker status, strict diet, and problems with walking or conducting physical activity in the prior 6 months. Subjects who consumed drugs to treat the clinical features of cardiovascular diseases (such as blood pressure-, lipid-, and/or glucose-lowering drugs) were also excluded from this study. Ethical clearance was obtained from the Medical and Health Research Ethics Committee (MHREC) Faculty of Medicine, Universitas Gadjah Mada, Indonesia (KE/FK/791/EC/2015). This study followed the ethical guidelines of the 1975 Declaration of Helsinki.

Obesity status was defined by body mass index (BMI) and calculated by dividing body weight with the square of height. Body composition was defined by percent body fat. Body weight and body fat were measured using a digital body mass scale and bioelectrical impedance (0.01 kg precision, Omron Karada Scan HBF-375, Osaka, Japan). Height was measured using a wall-mounted tape measure (0.1 cm precision, GEA medical, Jakarta, Indonesia). Waist and hip circumference were measured using a non-elastic tape (0.1 cm precision). All anthropometric measurements were done by trained personnel using calibrated instruments.

Blood pressure was measured using Omron HEM 7120 Automatic Blood Pressure (Omron, Japan). This measurement was done after at least 10 minutes rest from recent activity and the participants were asked to sit in a comfortable sitting position with their left arm fully exposed and resting on a supportive surface at the heart level. Blood pressures were measured on the left arm using appropriate cuff size.

Data on dietary intake were collected using a validated Semi-Quantitative Food Frequency Questionnaire (SQ-FFQ) and the analysis was based on Indonesian food database and United States Department of Agriculture [22]. Data of habitual consumption of food items that were collected using the SQ-FFQ were translated into daily intake [23]. Data collection for dietary intake was done by a face-to-face interview between trained nutritionists and subjects.

The Dietary inflammatory Index (DII®) score is a calculated parameter that gives an overall picture of the inflammatory properties of the diet. An individual's diet is considered more pro-inflammatory when the DII score is more positive, while the diet is considered more anti-inflammatory when the DII score is more negative. The DII score was calculated according to Shivappa et al. [9]. The calculation of the dietary inflammatory index score was based on 30 nutrients and food components including total energy, protein, carbohydrate, total fat, saturated fat, trans fat, mono-unsaturated fatty acid (MUFA), poly-unsaturated fatty acid (PUFA), omega-3 fatty acid, omega-6 fatty acid, cholesterol, fibre, magnesium, iron, selenium, zinc, vitamin A, vitamin C, vitamin D, vitamin E, thiamin, riboflavin, vitamin B6, vitamin B12, folate, niacin, beta-carotene, alcohol, caffeine, and tea. No data were available for the other dietary factors included by Shivappa et al. [9] in their dietary inflammation index such as rosemary, oregano, pepper, turmeric, saffron, onion, ginger, garlic, and polyphenols (i.e., isoflavones and anthocyanidins). To derive the individual DII scores, the global average intake (taken from Shivappa et al. [9]) was subtracted from the reported daily intake of each nutrient in the FFQ and divided by the standard deviation of the global daily intake, rendering a z-score which was converted into a centered percentile score. This score was then multiplied with an overall inflammatory effect score. All 30 (out of 48) included individually calculated nutrient-specific effect scores were then summed to obtain the DII score.

Data on physical activity was collected using the International Physical Activity Questionnaire (IPAQ) [24]. This questionnaire contains information on the intensity and duration of several activities including work/job, transportation, house-related work and maintenance, recreation, exercise and leisure-time physical activity. Each activity has a unique metabolic equivalent of task (MET) score, which represents the amount of energy used for a certain type of activity. In order to obtain an overall picture of the individual's physical activity, all the activities that have been reported in IPAQ are transformed into MET-minutes/week. The SQ-FFQ and IPAQ were developed, validated and used before [23–25].

From each participant a 10-mL blood sample was collected in ethylenediaminetetraacetic acid (EDTA)-containing tubes. After collection, blood plasma and buffy coat were separated by centrifugation. Plasma HDL cholesterol

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and triglyceride concentration were measured using the cholesterol oxidase phenol 4-aminoantipyrine peroxidase (CHOD-PAP) and glycerol phosphate oxidase (GPO) methods, respectively (Diasys, Holzheim, Germany). Plasma leptin concentration was measured using an enzyme-linked immunosorbent assay (DRG, Springfield Township, NJ, USA).

Statistical analyses were conducted using JASP (University of Amsterdam, the Netherlands) [26]. The relationships between the DII score as independent variable and body weight, blood pressure, lipid profile and leptin concentration as dependent variables were analyzed by linear regression analysis (Model 0, unadjusted). Adjustments for age, sex, physical activity and energy intake, and BMI where relevant, were done. Model I represents a regression analysis with adjustment for age, sex, and BMI (for blood pressure, triglycerides, HDL and leptin). Model II represents a regression analysis with adjustment for age, sex, energy intake, physical activity and BMI (for blood pressure, triglycerides, HDL and leptin). In addition, we made groups based on tertiles of the DII score and compared the variables by ANOVA (analysis of variance) or ANCOVA (Analysis of covariance). The ANCOVA analysis adjusted for age, sex, energy intake, and physical activity when analyzing differences in anthropometric measures among DII score tertiles. For analysis of blood pressure, lipid profile, and leptin differences between DII score tertiles, ANCOVA adjusting for age, sex, body mass index, energy intake, and physical activity was used.

RESULTS

Characteristics of study participants are shown in Table 2.1. In this study, 503 adults (men 50.1% and women 49.9%) living in Yogyakarta were investigated. Data on the characteristics of men and women separately can be found in Supplementary Table 2.1. Data of dietary intake of study participants are shown and compared with global data of dietary intake based on Shivappa et al. [9] (Table 2.2). Compared to global averages, participants in this study had a relatively high intake of pro-inflammatory components such as total energy, carbohydrate, iron, and vitamin B12. They had a relatively low intake of anti-inflammatory components such as omega 3 fatty acid, omega 6 fatty acid, niacin, vitamin A, vitamin D, vitamin E, alcohol, and caffeine, but a relatively high intake of other anti-inflammatory components such as fiber, magnesium, selenium, vitamin C, folic acid, and tea.

Table 2.1. Characteristics of study participants.

	All (n=503)
Age (years)	41.6 ± 10.2
Anthropometric measures	
Body weight (kg)	62.6 ± 13.1
Height (cm)	158.5 ± 9.0
Body mass index (kg/m ²)	24.9 ± 5.0
Body fat (%)	27.5 ± 8.7
Waist circumference (cm)	86.2 ± 12.7
Hip circumference (cm)	93.9 ± 11.2
Blood pressure	
Systolic (mmHg)	128.6 ± 25.2
Diastolic (mmHg)	79.7 ± 14.8
Metabolic profile	
Triglycerides (mmol/L)	1.57 ± 0.82
HDL (mmol/L)	1.18 ± 0.47
TG/HDL ratio	1.48 ± 1.02
Leptin (ng/mL)	7.3 ± 8.8
Physical activity (METS-min./week)	5781 ± 5932
Dietary intake	
Energy (kJ/day)	10 838 ± 4 789
Protein (%)	12.5 ± 3.4
Fat (%)	23.1 ± 10.6
Carbohydrate (%)	64.3 ± 10.8
Dietary inflammatory index	1.01 ± 7.29
% overweight^a	45.9
% obese^b	14.9

Values are presented as mean ± standard deviation, *p*. value of difference between men and women (2-sample t-test), ^abody mass index ≥ 25 kg/m² or higher, ^bbody mass index ≥ 30 kg/m² or higher, ^{a,b}percent in comparison to total study participants, * independent t-test, ^Mann-withney test, #Chi-square test.

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Table 2.2. Comparison of global dietary intake and dietary intake of Indonesian adults.

	Global dietary intake	Dietary intake of study participants
Pro-inflammatory components		
Carbohydrate (g)	272.2 ± 40.0	416.9 ± 202.8
Cholesterol (mg)	279.4 ± 51.2	177.8 ± 161.8
Energy (kj)	8 606 ± 1 414	10 838 ± 4 789
Iron (mg)	13.35 ± 3.71	18.1 ± 9.8
Protein (g)	79.4 ± 13.9	80.8 ± 41.9
Saturated fat (g)	28.6 ± 8.0	25.7 ± 19.6
Total fat (g)	71.4 ± 19.4	66.6 ± 45.9
Trans fatty acid (g)	3.15 ± 3.75	2.82 ± 4.72
Vitamin B ₁₂ (µg)	5.15 ± 2.70	7.38 ± 9.54
Anti-inflammatory components		
Alcohol (g)	19.98 ± 3.72	0.03 ± 0.07
Beta - carotene (µg)	3 718 ± 1 720	3 696 ± 3 721
Caffeine (g)	8.05 ± 6.67	0.09 ± 0.10
Fiber (g)	18.8 ± 4.0	24.3 ± 13.5
Folic acid (µg)	273.0 ± 70.7	403.0 ± 339.4
Magnesium (mg)	310.1 ± 139.4	392.1 ± 278.2
MUFA (g)	27.0 ± 6.1	29.5 ± 23.1
Niacin (mg)	25.9 ± 11.8	19.57 ± 9.14
Omega 3 fatty acids (g)	1.06 ± 1.06	0.46 ± 0.47
Omega 6 fatty acids (g)	10.80 ± 7.50	0.86 ± 0.80
PUFA (g)	13.88 ± 3.76	13.40 ± 9.64
Riboflavin (mg)	1.70 ± 0.79	1.97 ± 1.72
Selenium (µg)	67.0 ± 25.1	103.0 ± 49.6
Black/green tea (g)	1.69 ± 1.53	2.36 ± 2.60
Thiamin (mg)	1.70 ± 0.66	1.39 ± 0.71
Vitamin A (RE)	983.9 ± 518.6	574.3 ± 508.0
Vitamin B ₆ (mg)	1.47 ± 0.74	4.59 ± 3.71
Vitamin C (mg)	118.2 ± 43.5	152.3 ± 145.5
Vitamin D (µg)	6.26 ± 2.21	3.44 ± 5.88
Vitamin E (mg)	8.73 ± 1.49	2.74 ± 2.65
Zinc (mg)	9.84 ± 2.19	9.59 ± 4.75

Data on the relationship between the DII score and anthropometric measures are shown in Table 2.3. We found that the DII score was not associated with any of the anthropometric measures (all $p > 0.05$). This remained after adjustment for age, sex, physical activity and energy intake (Table 2.3). The DII score was also not associated with systolic and diastolic blood pressure, triglycerides and HDL cholesterol

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(all $p > 0.05$). These associations also remained non-significant after adjustment for covariates (Table 2.3). However, after correction for age, sex, BMI, energy intake, and physical activity we found that the DII score was positively associated with plasma leptin concentration (Table 2.3). The sex specific analyses of the relationship between the DII score and all measured parameters are shown in Supplementary Table 2.2. There were no sex-related differences in the relationships between the DII score and any of the measured parameters.

Table 2.3. Standardized regression coefficients (B) and their standard error (SE) and p -value of the association between DII score and anthropometric variables, blood pressure, lipid profile, and leptin concentration.

Variables	Model 0 ^a			Model I ^b			Model II ^c		
	B ^a	SE ^a	p ^a	B ^b	SE ^b	p ^b	B ^c	SE ^c	p ^c
Body weight (kg)	-0.010	0.081	0.900	-0.011	0.078	0.801	-0.039	0.096	0.470 ^
BMI (kg/m ²)	-0.012	0.031	0.693	-0.020	0.030	0.648	-0.080	0.036	0.132 ^
Body fat (%)	-0.018	0.054	0.736	-0.009	0.035	0.762	-0.044	0.043	0.222 ^
Waist circumference (cm)	-0.079	0.078	0.311	-0.048	0.074	0.251	-0.046	0.091	0.379 ^
Hip circumference (cm)	0.012	0.069	0.857	0.006	0.064	0.878	-0.049	0.079	0.347 ^
Systolic BP (mmHg)	0.221	0.154	0.153	0.066	0.134	0.088	0.034	0.165	0.481 *
Diastolic BP (mmHg)	0.128	0.091	0.159	0.065	0.082	0.107	0.046	0.102	0.355 *
Triglycerides (mmol/L)	-0.004	0.005	0.407	-0.037	0.005	0.388	0.045	0.006	0.406 *
HDL cholesterol (mmol/L)	0.0003	0.003	0.925	0.010	0.003	0.825	-0.046	0.004	0.400 *
Leptin (ng/mL)	0.101	0.061	0.102	0.105	0.042	0.002	0.096	0.052	0.020 *

^a Model 0, linear regression analysis without adjustment; ^b Model I, linear regression analysis with adjustment for age and sex; ^c Model II, linear regression analysis with correction for age, sex, energy intake, physical.

To further clarify the association between DII score, anthropometric measures, blood pressure, lipid profile and leptin, we divided subjects into three tertiles based on their DII score (Table 2.4). No significant differences among the 3 tertiles (ANOVA, all $p > 0.05$) were found. However, after adjustment for age, sex, energy intake, physical activity and body mass index, the increase in leptin concentration across DII score tertiles was statistically significant (ANCOVA, $p = 0.031$).

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Table 2.4. The differences on anthropometric measures, lipid profile, blood pressure and leptin among DII score tertiles.

Variables	DII Score Tertile 1 (<-1.0) (n = 169)	DII Score Tertile 2 (1.0-5.1) (n = 168)	DII Score Tertile 3 (>5.1) (n = 164)	<i>P</i> _{ANOVA} *	<i>P</i> _{ANCOVA}
Anthropometric measures					
Body weight (kg)	61.9 ± 13.9	63.5 ± 11.0	62.3 ± 14.2	0.538	0.840 ^
Body mass index (kg/m ²)	24.7 ± 4.8	25.3 ± 4.7	24.9 ± 5.5	0.523	0.470 ^
Body fat (%)	27.4 ± 8.6	27.5 ± 8.7	27.7 ± 9.0	0.937	0.543 ^
Waist circumference (cm)	86.2 ± 12.7	87.1 ± 12.4	85.4 ± 13.2	0.467	0.395 ^
Hip circumference (cm)	93.1 ± 10.3	95.0 ± 10.7	93.7 ± 12.5	0.301	0.209 ^
Systolic blood pressure (mmHg)	126.9 ± 25.2	129.3 ± 24.5	129.5 ± 25.9	0.590	0.847 #
Diastolic blood pressure (mmHg)	79.1 ± 14.3	80.2 ± 14.4	79.9 ± 15.8	0.773	0.790 #
Triglycerides (mmol/L)	1.6 ± 0.9	1.6 ± 0.8	1.5 ± 0.8	0.479	0.413 #
HDL cholesterol (mmol/L)	1.2 ± 0.5	1.1 ± 0.4	1.2 ± 0.5	0.378	0.572 #
Leptin (ng/mL)	6.2 ± 6.9	7.1 ± 8.5	8.6 ± 10.7	0.071	0.031 #

* ANOVA analysis across DII score tertiles; ^ ANCOVA analysis across DII score tertiles with adjustment for age, sex, energy intake and physical activity; # ANCOVA analysis across DII score tertiles with adjustment for age, sex, body mass index, energy intake, and physical activity.

DISCUSSION

This study was aimed to evaluate the relationship between DII score, body weight, blood pressure, lipid profile and leptin in Indonesian adults. We found that DII score was not correlated with any of the anthropometric measures, blood pressure or lipid profile. Interestingly, we showed that the DII was positively correlated with plasma leptin concentration after correction for age, sex and BMI, energy intake and physical activity.

We compared the intake of the components of the DII in our Indonesian population with the global averages described by Shivappa [9]. Participants in this study had higher intake of pro-inflammatory components such as total energy, carbohydrates, iron and vitamin B12 and a lower intake of anti-inflammatory components such as omega 3 fatty acids, omega 6 fatty acids, niacin, vitamin A, vitamin D, and vitamin E. The mean energy intake of men was 11,285 kJ/day and this matches the Indonesian dietary recommendation for male adults (11,406 kJ/day) [27]. The mean energy intake of women was 10,277 kJ/day and this was slightly

higher than the Indonesian dietary recommendation for female adults (9418 kJ/day) [27]. Results from this study provide a practical implication that can help reduce the inflammatory properties of diet of individuals in the study population. This can be done by reducing the consumption of carbohydrate rich foods (e.g., rice, sugar and wheat-based products) and increasing consumption of unsaturated fat and protein rich foods (such as eggs and fatty fish). This is because, although protein is considered as pro-inflammatory nutrient, it has a lower inflammatory effect score than carbohydrate [9]. Additionally, unsaturated fats are anti-inflammatory nutrients and foods that are rich in unsaturated fats usually also contain fat soluble vitamins such as vitamin A, D, and E [22]. Those vitamins were reported to be lower in these study participants compared to the global dietary intake.

This study was initiated by our earlier finding that in overweight/obese Caucasian men and women who undertook a weight loss program, the DII score was correlated with weight regain during follow-up [15]. In this study we showed that the DII score was not correlated with body weight in Indonesian adult men and women. The relationship between the DII score and obesity indices has also been evaluated elsewhere. In a cross-sectional analysis of the Spanish PREDIMED trial, the DII score was associated with BMI only in women but not in men [13]. In Myanmar, a small cross-sectional study among overweight and non-overweight female school teachers showed that the DII score was not associated with overweight [21]. A role for DII in the development of obesity was indicated by Ramallal et al. [14]. They showed that in a non-overweight adult Spanish cohort the DII score was not associated with BMI at baseline, but a higher DII score was associated with a higher body weight increment after 8 years of follow up and a higher risk of developing overweight or obesity. Based on these studies and our findings, the effect of the DII on body weight remains inconclusive. Well-controlled longer-term intervention studies are required to shed more light on the role of DII in body weight regulation.

There is more convincing evidence that inflammation is an important link between obesity and its cardiovascular co-morbidities [3–6]. Previously it was suggested that low-grade inflammation was associated with the development of dyslipidemia [3]. In this study, we found that the DII score was not correlated with components of the lipid profile, such as triglycerides and HDL. In contrast, Neufcourt et al. showed in a large (n = 3 726) cohort of French adults [18] that at baseline, the DII score was positively associated with triglyceride level, but not with HDL cholesterol level. After a follow up of 13 years, the DII score was significantly associated with higher triglyceride and lower HDL cholesterol levels. The association between the DII score and lipid profile was also confirmed by a small scale study (n = 90) in Colombia [19]. These investigators showed that study participants with a

higher pro-inflammatory diet (based on DII score calculation) had a significantly lower HDL level. Our intermediate size study in Indonesian adults could not confirm these findings. On the other hand, there was also no increased risk for low HDL cholesterol or elevated triglyceride concentrations with increasing DII score in a study in 447 U.S. police officers [28]. Dyslipidemia or disturbance of lipid profile including triglyceride and cholesterol levels have long been used as an early biomarker of cardiovascular diseases. However, this concept is now being challenged. Inflammation may be a more important biomarker of cardiovascular diseases [16]. This shift of paradigm is necessary because this might affect dietary approaches for cardiovascular diseases prevention. Instead of aiming to lower cholesterol and triglycerides, it might be more beneficial to lower the inflammatory properties of the diet as a means of prevention of cardiovascular diseases.

In this study we also showed that the DII score was not associated with blood pressure, neither in men nor women. This finding differs from studies previously reporting that the DII score was associated with increased risk for developing hypertension among middle-aged Australian women [20] and Polish adults [29]. On the other hand, Wirth et al. [28] also found no higher risk of elevated blood pressure with increasing DII score in U.S. police officers.

We cannot exclude the possibility that the lack of association between the DII score and lipid profile and blood pressure is due to our inclusion criteria. We only included subjects without a clinical diagnosis cardiovascular diseases, diabetes, or treatment for dyslipidemia or high blood pressure, because we were interested in investigating the role of diet in the early onset development of chronic diseases and thus our population can be regarded as relatively healthy compared to the general Indonesian population.

In this study, we showed that the DII score was positively associated with plasma leptin concentration after adjustment for age, sex, BMI, energy intake, and physical activity. This finding was confirmed by analysis based on DII score tertiles, which showed that leptin concentration increased significantly across the tertiles. Leptin is produced by adipose tissue and its production increases with the progression of adiposity [30]. In the past few decades, there has been a growing interest in understanding the interaction between leptin, inflammation and oxidative stress. Leptin is a cytokine which is produced by adipocytes with an ability to induce inflammation. The pro-inflammatory properties of leptin have been suggested to be similar to those of immune cell-derived cytokines such as tumor necrosis factor alpha (TNF-alpha) and interleukin 6 (IL-6) [31,32]. In addition to inflammation, it was suggested that leptin plays an important role in the development of oxidative stress in obesity by inducing production of reactive intermediates such as H₂O₂ and hydroxyl radicals [33]. The accumulation of these processes can induce development

of cardiovascular diseases. This is supported by several studies, which showed that a higher leptin concentration was associated with increased risk for cardiovascular diseases [34–36]. To our knowledge, only one other study has investigated the relationship between DII score and leptin [28]. In this cross-sectional study in U.S. police officers, the DII score was not associated with leptin concentration.

There are several strengths and limitations in this study. The strengths of this study are the relatively large sample size and the non-Western population. However, there are several limitations to this study. First, because data collection was done using a SQ FFQ for Indonesian food consumption with limited data on bioactive components of foods, we cannot provide data on the intake of a number of herbs/seasonings (such as oregano, rosemary, pepper, saffron, garlic, onion, and ginger) as well as polyphenols (such as flavonol, anthocyanidins, and eugenol), which are included in the original list provided by Shivappa et al. [9]. Additionally, there are some food components that have no known value for different types of PUFAs in the food databases used, which might have an impact on total omega 3 and 6 consumption. These might influence the relationships between DII score and outcomes in comparison to the full DII according to Shivappa et al. Second, we reported data on dietary intake amongst individuals who live in urban areas. Therefore, data from this study may not represent the intake of those that live in rural areas. Third, because of the design of this study, no conclusion about causality can be drawn. Fourth, measurements were performed at random times during the day and in the non-fasting state, which may have interfered with the potential association between the DII score and the outcome variables by increasing the variation. However, it was previously shown that in large cohorts the influence of normal food intake on changes in lipids, lipoproteins, and apolipoproteins is small and that the random non-fasting lipid profile remains a good predictor for cardiovascular diseases in humans [37]. However, leptin production has a diurnal variation [38] and is affected by fasting status [39], and therefore confirmation of our findings under better standardized conditions is needed.

In summary, we showed that the DII score was positively correlated with the plasma leptin concentration, which is one of the markers of adipose tissue inflammation. This might explain the connection between the DII score and increased systemic inflammation as well as cardiovascular diseases in other studies. Because of the potential importance of diet in the development of inflammation, intervention studies that investigate the effect of manipulation of the inflammatory properties of the diet on the inflammatory process are warranted.

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Author contributions

Conceptualization, H.F.L.M., D.C.S., E.H., YY.L., and W.A.M.W.M.; Methodology, H.F.L.M., D.C.S., E.H., YY.L., and W.A.M.W.M.; Software, H.F.L.M.; Validation, H.F.L.M., D.C.S., and E.H.; Formal Analysis, H.F.L.M., M.A.v.B., and E.C.M.; Investigation, H.F.L.M., D.C.S., E.H., YY.L., and W.A.M.W.M.; Resources, H.F.L.M. and W.A.M.W.M.; Data Curation, H.F.L.M., M.A.v.B., and E.C.M.; Writing—Original Draft Preparation, H.F.L.M., M.A.v.B., and E.C.M.; Writing—Review and Editing, H.F.L.M., M.A.v.B., and E.C.M.; Visualization, H.F.L.M., M.A.v.B., and E.C.M.; Supervision, W.A.M.W.M.; Project Administration, H.F.L.M., and YY.L.; Funding Acquisition, H.F.L.M., and W.A.M.W.M.

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Conflicts of Interest

The authors declare no conflict of interest.

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CHAPTER 2

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CHAPTER 2**Supplementary Table 2.1.** The comparison of anthropometric measures, blood pressure, lipid profile, dietary intake, physical activity and leptin between men and women.

	Men (n=252)	Women (n=251)	p
Age (years)	41.1 ± 10.8	42.1 ± 9.6	0.500 [^]
Anthropometric measures			
Body weight (kg)	65.4 ± 13.2	59.7 ± 12.3	<0.001 [^]
Height (cm)	164.9 ± 6.4	152.0 ± 6.2	<0.001 [*]
Body mass index (kg/m ²)	24.0 ± 4.4	25.9 ± 5.4	<0.001 [^]
Body fat (%)	21.1 ± 6.0	33.9 ± 5.9	<0.001 [^]
Waist circumference (cm)	84.4 ± 11.8	88.1 ± 13.4	0.003 [^]
Hip circumference (cm)	91.4 ± 9.8	96.5 ± 11.9	<0.001 [^]
Waist-to-hip ratio	0.92 ± 0.06	0.91 ± 0.07	0.094 [^]
Blood pressure			
Systolic (mmHg)	131.0 ± 26.5	126.3 ± 23.7	0.020 [^]
Diastolic (mmHg)	81.2 ± 15.8	78.2 ± 13.6	0.043 [^]
Metabolic profile			
Triglycerides (mmol/L)	1.71 ± 0.91	1.42 ± 0.67	0.003 [^]
HDL cholesterol (mmol/L)	1.07 ± 0.36	1.30 ± 0.54	<0.001 [^]
Leptin (ng/mL)	2.7 ± 4.8	11.4 ± 9.6	<0.001 [^]
Physical activity (METS-min/week)	6815 ± 6532	4747 ± 5071	<0.001 [^]
Dietary intake			
Energy (kJ/day)	11 394 ± 4 621	10 277 ± 4 898	0.002 [^]
Protein (energy%)	12.5 ± 3.3	12.6 ± 3.5	0.664 [^]
Fat (energy%)	21.3 ± 9.8	25.0 ± 11.1	<0.001 [^]
Carbohydrate (energy%)	66.1 ± 9.9	62.5 ± 11.4	<0.001 [^]
Dietary Inflammatory Index score	1.07 ± 7.19	0.95 ± 7.4	0.898 [^]
% overweight ^a	19.7	26.2	0.003 [#]
% obese ^b	3.6	11.3	<0.001 [#]

Values are presented as mean ± standard deviation, p value of difference between men and women, ^abody mass index ≥ 25 kg/m² or higher, ^bbody mass index ≥ 30 kg/m² or higher, ^{a,b}percent of total study participants, * independent t-test, [^]Mann-Whitney test, [#]Chi-square test.

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Supplementary Table 2.2. Linear regression analysis with the DII score as independent variable for men and women.

	Men (n=252)						Women (n=251)					
	Model 0		Model I		Model II		Model 0		Model I		Model II	
	B	p	B	p	B	p	B	p	B	p	B	p
Body weight (kg)	-0.157	0.180	-0.082 ^a	0.194	-0.037 ^b	0.643	0.121	0.249	0.062 ^a	0.323	-0.020 ^b	0.787
BMI (kg/m ²)	-0.049	0.204	-0.076 ^a	0.224	-0.049 ^b	0.535	0.025	0.593	0.022 ^a	0.720	-0.084 ^b	0.257
Body fat (%)	-0.040	0.446	-0.042 ^a	0.487	-0.026 ^b	0.731	0.023	0.650	0.015 ^a	0.804	-0.075 ^b	0.295
Waist circumference (cm)	-0.127	0.227	-0.071 ^a	0.245	-0.011 ^b	0.891	-0.031	0.789	-0.033 ^a	0.578	-0.058 ^b	0.414
Hip circumference (cm)	-0.082	0.345	-0.054 ^a	0.379	-0.036 ^b	0.638	0.107	0.294	0.053 ^a	0.381	-0.039 ^b	0.596
Systolic BP (mmHg)	0.173	0.460	0.049 ^a	0.424	0.056 ^c	0.440	0.261	0.196	0.066 ^a	0.244	0.005 ^c	0.938
Diastolic BP (mmHg)	0.069	0.622	0.034 ^a	0.589	0.052 ^c	0.485	0.181	0.120	0.086 ^a	0.156	0.039 ^c	0.574
Triglycerides (mmol/L)	-0.009	0.263	-0.067 ^a	0.300	-0.059 ^c	0.448	-0.0002	0.973	-0.008 ^a	0.896	-0.044 ^c	0.573
HDL cholesterol (mmol/L)	-0.005	0.160	-0.096 ^a	0.136	-0.131 ^c	0.102	0.006	0.230	0.084 ^a	0.198	0.003 ^c	0.971
Leptin (ng/mL)	0.055	0.281	0.081 ^a	0.263	0.157 ^c	0.019	0.160	0.070	0.120 ^a	0.078	0.110 ^c	0.089

Model 0, linear regression analysis without adjustment; Model I and Model II, linear regression analysis with adjustment; ^alinear regression analysis with adjustment for age; ^blinear regression analysis with adjustment for age, energy intake and physical activity; ^clinear regression analysis with adjustment for age, sex, body mass index, energy intake and physical activity. BP: blood pressure.

CHAPTER 3

Dietary Intake after Weight Loss and the Risk of Weight Regain: Macronutrient Composition and Inflammatory Properties of the Diet

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Abstract

Weight regain after successful weight loss is a big problem in obesity management. This study aimed to investigate whether weight regain after a weight loss period is correlated with the macronutrient composition and/or the inflammatory index of the diet during that period. Sixty one overweight and obese adults participated in this experimental study. Subjects lost approximately 10% of their initial weight by means of very low-calorie diet for five weeks, or a low calorie diet for 12 weeks. After that, subjects in both groups followed a strict weight maintenance diet based on individual needs for four weeks, which was followed by a nine-month weight maintenance period without dietary counseling. Anthropometrics and dietary intake data were recorded before weight loss (baseline) and during the weight maintenance period. On average, participants regained approximately half of their lost weight. We found no evidence that macronutrient composition during the weight maintenance period was associated with weight regain. The dietary inflammatory index ($r = 0.304$, $p = 0.032$) was positively correlated with weight regain and remained significant after correction for physical activity ($r = 0.287$, $p = 0.045$). Our data suggest that the inflammatory properties of diet play a role in weight regain after weight loss in overweight and obese adults.

Keywords: obesity; weight regain; macronutrient composition; inflammation

Introduction

It has been estimated that a total of 107.7 million children and 603.7 million adults worldwide were obese in 2015 [1], and this state of over-nutrition was responsible for an increasing economic and health burden [1,2]. Effective actions to prevent the increasing rate of obesity and to treat those who already are obese are required. Lifestyle-based weight loss programs, which include an energy-restricted diet and increased physical activity, are by far the most commonly used weight loss methods. Effective lifestyle-based weight loss interventions have been developed in the last few decades, but maintaining the attained weight loss is not an easy task [3]. Studies have shown that overweight and obese individuals who lost weight through lifestyle modification are prone to weight regain [4–6].

Several theories have been proposed to explain this weight regain in which biologic and behavioral factors play an important role [7]. Reduction in basal and activity-related energy expenditure, changes in hunger- and satiety-associated hormone production, and an increase in fat cell stress in response to fat mass reduction have been suggested as potential factors associated with the tendency towards gaining weight after weight loss [7,8]. In addition, several eating-related behavioral factors, such as ability to control over-eating, binge eating and eating as a response to negative emotions have also been suggested to increase the risk of weight regain [9].

Several studies have been conducted to evaluate whether dietary factors are associated with weight regain. Several studies found that higher dietary protein content after weight loss was associated with less weight regain [10–14]. However, not all studies support this notion [15,16]. The role of other macronutrients in the prevention of weight regain has been less well studied.

Dietary intake not only has a direct impact on body weight, but may also have indirect effects that are important for body weight regulation. We have previously shown that weight regain after weight loss was correlated with the expression of genes related to adipose tissue stress and inflammation [17,18]. Because inflammation is also influenced by the dietary pattern [19], the inflammatory properties of the diet might play role in weight regain.

Therefore, this study aimed to investigate the influence of the macronutrient composition and inflammatory properties of the diet on weight regain during a weight maintenance period after weight loss of overweight and obese individuals. To analyze the correlation between the inflammatory properties of diet and weight regain we calculated the dietary inflammatory index (DII). The DII consists of a compilation of effects of intake of specific nutrients that have been shown to change inflammatory parameters in the blood, and provides a quantitative estimate of the inflammatory tendency of an individual's diet [19–22]. In addition, we analyzed the association between each individual component of the dietary inflammatory index and weight regain.

METHODS

Subjects

Male (n = 27, 45.8%) and female (n = 30, 54.2%) Caucasian adults with overweight and obesity participated in this study. The recruitment process was done through advertisements. The inclusion criteria were body mass index more than 28 kg/m² with stable body weight in the past 2 months prior to the study. Those who had a current or previous history of cardiovascular disease, type 2 diabetes mellitus, liver or kidney disease, used medication that influences body weight regulation, were pregnant, smoking, or had marked alcohol consumption were excluded from the study. Subjects with elevated fasting glucose (>6.1 mmol/L), total cholesterol (>7.0 mmol/L) or triacylglycerol (>3.0 mmol/L) concentrations, or blood pressure (>160/100 mmHg) prior to the intervention were also excluded. A total of 61 subjects started this study and provided their written informed consent before participation. The study was performed according to the Declaration of Helsinki and was approved by the Medical Ethics Committee of Maastricht University Medical Centre. This trial is registered at www.clinicaltrials.gov as NCT01559415. This study is a secondary analysis of a study of which the design and methods have been described in detail before [23].

The Dietary Intervention Program

The dietary intervention program was composed of three periods: weight loss period (WL), weight stable period (WS) and follow-up period (FU). The weight maintenance (WM) period is a combination of the weight stable and follow-up periods. During WL, subjects were divided into two groups: a low calorie diet (LCD, 1250 kcal/day) group and a very low calorie diet (VLCD, 500 kcal/day) group. The LCD group replaced one meal by a meal replacement (Modifast; Nutrition et Sante Benelux, Breda, The Netherlands). The other two meals were prepared by the participants themselves based on meal plans designed by a dietitian, based on the Dutch national dietary guidelines, and they consumed three in-between meal snacks. The VLCD group consumed three meal replacements per day with additional 100 mL instant broth drinks per day and an unrestricted amount of low calorie vegetables. The length of the WL period was 12 weeks in the LCD group and 5 weeks in the VLCD group in order to achieve a similar 10% weight reduction in both groups. After the WL period, subjects in both groups followed a strictly balanced diet based on their individual energy requirements for 4 weeks (WS period) according to the Dutch national dietary guidelines [24]. During the follow-up period of 9 months, subjects were advised to maintain their body weight, but they no longer received dietary consultation and were free to choose their diet.

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Measurements

Anthropometric measurements were done at 4 time points: before the intervention (M1), at the end of WL (M2), at the end of WS (M3) and at the end of FU (M4) (Figure 3.1). Weight regain was calculated as body weight at M4 minus body weight at M3. Anthropometric measurements were obtained successfully at M1, M2 and M3 in all subjects but only 55 (96.5%) subjects were measured at M4 (Supplementary Table 3.1).

Height was measured using a stadiometer (Seca, Hamburg, Germany). Body weight was measured using a digital weighing scale (precision 0.1 kg) (Seca, Hamburg, Germany). Waist circumference was measured above the umbilicus and hip circumference was measured at the widest part of the buttocks. Percent body fat mass was determined by air displacement plethysmography (BodPod, Cosmed, Italy).

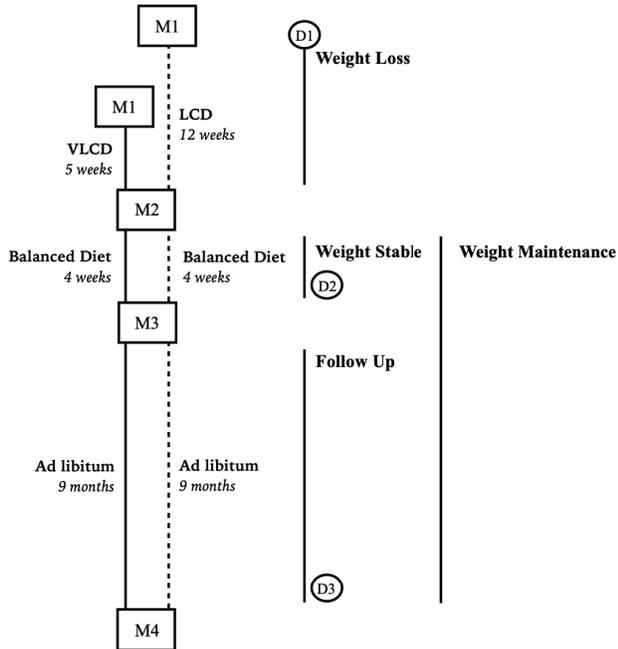


Figure 3.1. Schematic representation of study design and measurements. Anthropometric measurements M1: before intervention; M2: end of weight loss period; M3: end of weight stable period; M4: end of follow up period. Weight loss (WL) = M2 – M1; weight regain (WR) = M4 – M3. Baseline diet = D1, weight maintenance (WM) diet = average of D2 and D3. Physical activity was measured during M1, M2, M3 and M4.

Measurements of dietary intake were conducted at 3 time points: before the weight loss intervention (D1), at the end of WS (D2) and at the end of FU (D3) (Figure 3.1). At each time point, 3-day food diaries (including 1 non-working day) were collected. All foods reported were linked to the 2011 Dutch food consumption table (NEVO online version 2011, RIVM, Bilthoven, The Netherlands) and nutrient intakes were calculated. Some of the dietary intakes and physical activity differed significantly between D2 and D3 (Supplementary Table 3.2). However, because we do not know the time course of the changes from D2 to D3, we have assumed that the changes were gradual and the mean of D2 and D3 would best reflect the overall level during the follow-up period. Thus, the nutrient intake values obtained at D2 and D3 were averaged to reflect dietary intake over the weight maintenance (WM) period. Nutrients intakes were corrected for total energy intake [25] and a dietary inflammatory index was calculated according to Tabung et al. [26], based on the work of Shivappa et al. [20]. The calculation of the dietary inflammatory index was based on 27 nutrients including total energy, protein, carbohydrate, total fat, saturated fat, trans fat, mono-unsaturated fatty acid, poly-unsaturated fatty acid, omega-3 fatty acid, omega-6 fatty acid, cholesterol, fiber, alcohol, magnesium, iron, selenium, zinc, vitamin A, vitamin C, vitamin D, vitamin E, thiamin, riboflavin, vitamin B6, vitamin B12, folate and niacin, because no data were available for the other dietary factors included by Shivappa et al. [20] in their dietary inflammation index. Data on dietary intake during weight maintenance were obtained in 52 subjects (91.2%).

Subjects' total physical activity at baseline (M1), during the weight loss period (M2) and during weight maintenance period (M3 and M4) (Figure 3.1) was calculated using the sum score of occupational, leisure time and sports activity obtained from the Baecke questionnaire for habitual physical activity [27].

Data Analysis

Statistical analysis was performed with SPSS for Macintosh, Version 21 (Chicago, IL, USA). Data are presented as mean \pm standard error of the mean. Since no statistically significant differences were found between the VLCD and LCD groups, groups were combined for the correlation analyses. An independent t-test was used when comparing data on anthropometric, dietary and physical activity differences between the LCD and VLCD groups before and after the weight loss intervention. Paired t-tests were used when analyzing changes (anthropometric and dietary intake) within a group over a specific period of intervention. Wilcoxon signed ranks were used for non-normally distributed data. The correlation between nutrient intake, DII and weight regain was done using Pearson correlation tests for normally distributed data, and Spearman correlation tests for non-normally distributed data. Normality tests were performed using the Kolmogorov Smirnov normality test. In addition, partial correlations with correction for physical activity were determined. The analyses were considered statistically significant when $p < 0.05$ (2-tailed).

RESULTS

A total of 61 obese subjects were initially recruited in this study, but four subjects withdrew because of health and personal circumstances not related to the study. Data on anthropometric measures are presented in Table 3.1. Gender was equally distributed (LCD group male = 48.3%, VLCD group male = 46.4%). There were no differences in body weight and other anthropometric measures at any time point (M1, M2, M3 and M4) between subjects in LCD and VLCD groups (Supplementary Table 3.1). Subjects significantly lost weight at the end of the weight loss period (M2 vs. M1) and gained weight at the end of follow up period (M4 vs. M3) (Table 3.1). Weight regain varied between -3.8 kg and $+13.5$ kg.

Table 3.1. Changes in anthropometric data throughout the weight loss intervention.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.01$ paired sample t-test, change from M1 vs. M2, M2 vs. M3, and M3 vs. M4.

	Baseline (M1)	Weight Loss Period (M2)	Weight Stable Period (M3)	Follow Up Period (M4)
Age (years)	51.3 ± 9.1			
Height (cm)	172.3 ± 8.9			
Weight (kg)	92.5 ± 9.9	83.9 ± 1.2***	83.7 ± 9.5	88.3 ± 10.2***
BMI (kg/m ²)	31.2 ± 2.3	28.3 ± 0.3***	28.2 ± 2.4	29.6 ± 2.7***
Fat mass (%)	39.8 ± 8.6	34.8 ± 1.4***	33.8 ± 10.3***	36.3 ± 9.8***
Fat free mass (%)	60.2 ± 8.6	65.3 ± 1.4***	66.2 ± 10.3***	63.7 ± 9.8***
Fat mass (kg)	36.4 ± 7.8	28.9 ± 1.2***	27.9 ± 8.8***	31.7 ± 9.0***
Fat free mass (kg)	55.7 ± 11.5	54.6 ± 1.5***	55.2 ± 11.6***	56.0 ± 11.6*
Waist circumference (cm)	102.3 ± 9.6	94.7 ± 1.2***	94.63 ± 9.0	98.1 ± 9.2***
Hip circumference (cm)	111.0 ± 6.2	105.5 ± 0.9***	104.7 ± 6.3*	106.2 ± 8.3**

There were no differences in energy intake and dietary composition between the LCD and VLCD group before and during WM (all $p > 0.05$) (Supplementary Table 3.2) (Table 3.2). Subjects in both groups had a significantly lower total energy intake during WM ($p < 0.001$) compared to their initial energy intake. Protein and carbohydrate intake (as percent of total energy intake) increased (all $p < 0.05$) with no change in % energy from sugar consumption, whereas percentage of energy from fat was reduced, mainly due to a reduction in saturated fat (all $p < 0.05$). Alcohol consumption (expressed as g/1000 kcal) did not change significantly over the intervention period. The intake of fiber and several micronutrients (niacin, riboflavin, vitamin B6, folate, vitamin C, vitamin A, zinc, selenium, iron and magnesium), expressed as g or mg/1000 kcal, was increased during the weight maintenance period. In addition, the dietary inflammatory index during the weight maintenance period was lower than the baseline ($p < 0.001$). There was no change in physical activity ($p = 0.437$) over the intervention period.

CHAPTER 3

The correlations between macronutrients, the dietary inflammatory index and its components, and weight regain are shown in Table 3.3. No significant correlations with the intake of the various macronutrients were found. The dietary inflammatory index and total energy intake were positively correlated with weight regain, while magnesium, riboflavin and folate intake were negatively correlated with weight regain. Gender ($r = 0.036$, $p = 0.794$), body mass index ($r = -0.106$, $p = 0.439$), age ($r = 0.187$, $p = 0.171$) and initial weight loss ($r = 0.008$, $p = 0.955$) were not correlated with weight regain, while physical activity ($r = -0.278$, $p = 0.040$) was negatively correlated. Therefore, partial correlation analyses were conducted to evaluate the correlations between dietary intake, dietary inflammatory index and weight regain, independent of physical activity. Energy and riboflavin intake, and the dietary inflammatory index remained significantly correlated with weight regain, but not magnesium and folate.

Table 3.2. Dietary intake and physical activity at baseline and during weight maintenance in the LCD and VLCD groups and both groups combined (All).

	Baseline Diet (D1)			Weight Maintenance (D2 and D3)			p^*
	LCD	VLCD	All	LCD	VLCD	All	
Energy (kcal/day)	1991 ± 116	2120 ± 99	2071 ± 82	1677 ± 96	1713 ± 88	1684 ± 65	<0.001
Protein (%) ^a	17.5 ± 0.6	17.7 ± 0.9	17.6 ± 0.6	19.7 ± 0.8	20.1 ± 0.8	20.1 ± 0.6	<0.001 ^c
Carbohydrate (%) ^a	43.3 ± 1.3	45.0 ± 1.5	43.9 ± 7.3	48.0 ± 1.3	45.8 ± 1.1	46.8 ± 6.2	0.012
Sugars (%) ^a	19.8 ± 1.3	20.3 ± 1.2	20.2 ± 6.5	21.6 ± 1.2	19.6 ± 1.1	20.4 ± 6.3	0.801
Fat (%) ^a	37.0 ± 1.3	35.1 ± 1.3	36.3 ± 6.8	30.2 ± 1.0	32.3 ± 1.1	31.7 ± 5.4	<0.001
Saturated fat (g/ 1000 kcal) ^b	15.2 ± 0.8	14.6 ± 0.8	15.1 ± 0.6	11.7 ± 0.5	12.7 ± 0.4	12.2 ± 0.4	<0.001
Trans fat (g/ 1000 kcal) ^b	1.17 ± 0.09	1.00 ± 0.08	1.08 ± 0.06	0.85 ± 0.13	0.95 ± 0.07	0.91 ± 0.08	0.041 ^c
MUFA (mg/ 1000 kcal) ^b	13.8 ± 0.6	12.9 ± 0.5	13.5 ± 0.4	12.4 ± 0.7	12.4 ± 0.4	12.4 ± 0.4	0.104 ^c
PUFA (mg/ 1000 kcal) ^b	7.75 ± 0.51	7.67 ± 0.44	7.73 ± 0.34	6.85 ± 0.35	7.20 ± 0.36	7.05 ± 0.25	0.034 ^c
Omega 3 fatty acids (mg/ 1000 kcal) ^b	0.89 ± 0.09	0.75 ± 0.08	0.83 ± 0.06	0.90 ± 0.09	0.84 ± 0.06	0.87 ± 0.06	0.584 ^c
Omega 6 fatty acids (mg/ 1000 kcal) ^b	6.04 ± 0.52	6.26 ± 0.43	6.15 ± 0.35	5.27 ± 0.28	5.65 ± 0.33	5.48 ± 0.21	0.038
Cholesterol (mg/ 1000 kcal) ^b	118.1 ± 7.2	110.7 ± 8.1	117.2 ± 5.6	108.1 ± 8.4	106.3 ± 8.9	107.5 ± 6.1	0.202
Fiber (g/ 1000 kcal) ^b	9.77 ± 0.70	11.03 ± 0.64	10.48 ± 0.50	13.28 ± 0.55	13.88 ± 0.62	13.56 ± 0.42	<0.001
Alcohol (g/ 1000 kcal) ^b	3.17 ± 1.02	2.82 ± 1.04	3.12 ± 0.77	2.25 ± 0.59	2.30 ± 0.72	2.19 ± 0.46	0.185 ^c
Magnesium (mg/ 1000 kcal) ^b	145.8 ± 7.84	157.3 ± 6.1	152.2 ± 5.3	189.2 ± 6.7	182.3 ± 5.8	185.6 ± 4.6	<0.001
Iron (mg/ 1000 kcal) ^b	6.14 ± 0.39	5.89 ± 0.31	6.04 ± 0.26	7.16 ± 0.33	6.94 ± 0.21	7.07 ± 0.20	0.001 ^c
Selenium (mg/ 1000 kcal) ^b	27.8 ± 2.4	25.6 ± 2.0	26.8 ± 1.7	33.3 ± 2.4	28.6 ± 2.1	31.1 ± 1.6	0.037 ^c
Zinc (mg/ 1000 kcal) ^b	5.00 ± 0.22	5.33 ± 0.34	5.19 ± 0.21	5.88 ± 0.23	5.78 ± 0.31	5.86 ± 0.2	0.022 ^c
Vitamin A (µg/ 1000 kcal) ^b	362.9 ± 34.4	360.6 ± 41.5	373.2 ± 27.7	375.2 ± 28.2	534.6 ± 77.7	449.5 ± 40.9	0.046 ^c
Vitamin D (mg/ 1000 kcal) ^b	2.31 ± 0.42	1.77 ± 0.18	2.07 ± 0.25	1.99 ± 0.18	2.11 ± 0.25	2.05 ± 0.15	0.362 ^c
Vitamin E (mg/ 1000 kcal) ^b	6.58 ± 0.52	6.89 ± 0.49	6.79 ± 0.37	6.83 ± 0.39	6.48 ± 0.48	6.65 ± 0.31	0.667
Thiamin (mg/ 1000 kcal) ^b	0.64 ± 0.04	0.73 ± 0.06	0.69 ± 0.04	0.84 ± 0.05	0.72 ± 0.05	0.79 ± 0.04	0.037 ^c
Riboflavin (mg/ 1000 kcal) ^b	0.79 ± 0.07	0.78 ± 0.06	0.79 ± 0.04	0.99 ± 0.06	0.92 ± 0.07	0.97 ± 0.05	0.003 ^c
Vitamin B6 (mg/ 1000 kcal) ^b	0.89 ± 0.05	1.07 ± 0.08	0.99 ± 0.05	1.30 ± 0.08	1.25 ± 0.08	1.27 ± 0.06	<0.001 ^c
Folate (µg/ 1000 kcal) ^b	87.1 ± 10.3	95.8 ± 6.3	93.0 ± 6.4	128.1 ± 9.6	110.1 ± 6.9	119.7 ± 6.3	<0.001 ^c
Vitamin B12 (mg/ 1000 kcal) ^b	3.46 ± 0.96	2.26 ± 0.29	2.96 ± 0.55	3.27 ± 0.62	2.87 ± 0.44	3.04 ± 0.39	0.257 ^c
Niacin (mg/ 1000 kcal) ^b	9.28 ± 0.53	9.13 ± 0.61	9.15 ± 0.42	11.67 ± 0.68	10.81 ± 0.58	11.24 ± 0.46	0.001 ^c
Vitamin C (mg/ 1000 kcal) ^b	46.2 ± 7.6	54.0 ± 5.4	52.0 ± 4.9	67.4 ± 6.6	57.2 ± 5.5	61.2 ± 4.2	0.025 ^c
Dietary Inflammatory Index ^Δ	6.11 ± 0.43	5.73 ± 0.39	5.84 ± 0.31	4.44 ± 0.35	4.49 ± 0.35	4.48 ± 0.25	<0.001 ^c
Physical Activity [†]	9.07 ± 0.18	9.07 ± 0.21	8.95 ± 0.14	9.06 ± 0.20	8.78 ± 0.18	9.03 ± 0.14	0.437

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^aDietary intake as % of total energy intake; ^bdietary intake in gram or milligram per 1000 kcal of total energy intake; ^cwilcoxon signed ranks t-test for non-normally distributed data. *p value of paired t-test comparing baseline diet and weight maintenance diet in the whole group. [†]Dietary inflammatory index: The sum of dietary inflammatory scores of each nutrient as calculated by Tabung et al. [26]; [‡]physical activity was calculated using the Baecke questionnaire for habitual physical activity [27]. Data on dietary intake during the weight maintenance period are the average of dietary records obtained during the weight stable (WS) and the follow up (FU) periods; dietary intakes during WS and FU are provided in Supplementary Table 3.2; no significant differences were found between LCD and VLCD groups (independent t-test) before and during weight maintenance; LCD: low calorie diet group; VLCD: very low calorie diet group, MUFA: mono-unsaturated fatty acid; PUFA: poly-unsaturated fatty acid.

Table 3.3. Correlations between the dietary macronutrient content, the dietary inflammatory index and its components, and weight regain.

	Uncorrected Bivariate Correlation		Partial Correlation (corrected for physical activity)	
	r	p	r	p
Dietary Inflammatory Index [†]	0.304 ^b	0.032*	0.287	0.045*
Energy [‡] ^^	0.363 ^a	0.018*	0.344	0.027*
Protein [‡] ^^	-0.204 ^b	0.196	-0.212	0.183
Carbohydrate [‡] ^^	0.157 ^a	0.322	0.169	0.292
Sugars	0.043	0.786	0.058	0.721
Fat [‡] ^^	-0.054 ^a	0.735	-0.106	0.510
Saturated fat [‡] ^^	-0.035 ^a	0.824	-0.068	0.674
Trans fat [‡] ^^	-0.060 ^b	0.704	-0.090	0.577
MUFA [‡] ^^	-0.018 ^a	0.909	-0.059	0.714
PUFA [‡] ^^	-0.043 ^a	0.789	-0.070	0.663
Omega 3 fatty acids [‡] ^^	-0.095 ^a	0.551	-0.123	0.443
Omega 6 fatty acids [‡] ^^	-0.027 ^a	0.868	-0.065	0.684
Cholesterol [‡] ^^	-0.052 ^a	0.743	-0.060	0.711
Fiber [‡] ^^	-0.240 ^a	0.094	-0.170	0.242
Alcohol [‡] ^^	0.103 ^b	0.517	0.124	0.442
Magnesium [‡] ^^	-0.328 ^a	0.034*	-0.279	0.077
Iron [‡] ^^	-0.017 ^b	0.916	-0.092	0.566
Selenium [‡] ^^	-0.280 ^a	0.072	-0.277	0.080
Zinc [‡] ^^	-0.289 ^a	0.064	-0.253	0.111
Vitamin A [‡] ^^	0.002 ^b	0.990	-0.072	0.655
Vitamin D [‡] ^^	-0.026 ^b	0.873	0.025	0.878
Vitamin E [‡] ^^	-0.170 ^b	0.283	-0.273	0.084
Thiamin [‡] ^^	-0.199 ^b	0.207	-0.138	0.388
Riboflavin [‡] ^^	-0.387 ^a	0.011*	-0.378	0.015*
Vitamin B6 [‡] ^^	-0.229 ^a	0.144	-0.206	0.197
Folate [‡] ^^	-0.313 ^b	0.044*	-0.290	0.066
Vitamin B12 [‡] ^^	-0.125 ^b	0.429	-0.069	0.667
Niacin [‡] ^^	-0.130 ^a	0.413	-0.118	0.463
Vitamin C [‡] ^^	-0.230 ^b	0.142	-0.232	0.144

^aPearson correlation for normally distributed data; ^bSpearman correlation for non-normally distributed data; *p<0.05 (2-tailed); All nutrient intakes were expressed as the total amount corrected for energy intake per day. [‡]Energy intake was expressed in kcal/day; Data on dietary intake after weight loss is a combination on dietary intake during weight stable and follow up period; [†]Dietary inflammatory index: The sum of dietary inflammatory scores of each nutrient as calculated by Tabung et al [26];

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^^ Components of the dietary inflammatory index; MUFA: mono-unsaturated fatty acid; PUFA: poly-unsaturated fatty acid. Data on dietary macronutrient and DII were collected during weight maintenance period (average of D2 and D3), while weight regain was calculated as while weight regain is the different between follow up (M4) and weight stable (M3).

DISCUSSION

This experimental study evaluated the association of dietary intake with weight regain during a period of intended weight maintenance after successful weight loss. Subjects reduced their energy intake following a weight loss period, with no significant differences between the groups that had attained their weight loss within five or 12 weeks. The macronutrient composition of the weight maintenance diet was not associated with weight regain. In contrast, we showed that the dietary inflammatory index was positively correlated with weight regain, confirming that inflammation may play a role in the regulation of body weight after weight loss. Intake of several individual micronutrients with anti-inflammatory properties, such as magnesium, folate and riboflavin, were found to be negatively correlated with weight regain.

During the weight stable period, all subjects received dietary counseling to promote a healthy eating pattern based on the recommendations of the Netherlands nutrition centre [24]. According to this guideline, individuals should increase consumption of fruits, vegetables, whole grain cereals, and fatty fish. Those types of food provide a significant amount of dietary vitamins (such as thiamin, riboflavin and folate), minerals (such as magnesium and zinc), fiber and omega-3 fatty acids, which are considered as anti-inflammatory nutrients [20,28,29]. Reduction of the consumption of saturated fat and trans fat is also recommended, which will lower the dietary inflammatory index as well [20]. By adhering to these dietary guidelines, subjects should benefit from having a diet with lower dietary inflammatory index. Except for omega-3 fatty acid intake, intake of all the above nutrients showed significant changes in the recommended direction during the weight maintenance period.

Energy intake during the weight maintenance period, but not dietary macronutrient composition, was correlated with weight regain. This finding is in line with Sacks et al. [15] who showed that the long-term maintenance of weight loss depended on the reduced calorie intake irrespective of diet composition. However, this was different from data from the DiOGenes trial [10,11] and other smaller scale intervention studies [12,13,14], which showed that increased protein intake helps to maintain weight loss. In the present study, the only dietary advice given during the WM period was to follow the Dutch national recommendations, and thus, experimental manipulation of the diet composition was minor, resulting in a wide variation of dietary compositions in a relatively limited number of subjects, making it difficult to measure effects of individual macronutrients.

In this study, we found significant associations between the dietary inflammatory index and several of its components and weight regain. Based on our previous findings showing that weight regain was associated with continued weight-loss-induced adipocyte stress and inflammation [17,18], the hypothesis of this study was that a diet with a higher inflammatory index might also be associated with weight regain. The current results, indeed, seem to support this hypothesis. This is also supported by another study which showed that higher levels of inflammation markers in the systemic circulation (insulin, IL-6 and leukocyte number) and a higher adipose tissue inflammation were associated with resistance towards weight loss and proneness to weight regain [30]. The association between the dietary inflammatory index and obesity was previously reported in a cross-sectional study of adults [31]. This was confirmed by a large cohort study which showed that dietary inflammatory index can be used as a predictor of weight gain over eight years of follow-up, as well as of predisposition to the early development of obesity [32]. To our knowledge, this is the first study to show the effect of the dietary inflammatory index on weight regain in a weight loss trial. Overall, these results point to an important role of inflammation in the modulation of body weight, the development of obesity and the tendency for body weight regain after weight loss.

The dietary inflammatory index was based on a compilation of effects of specific nutrients and bioactive components that are derived from dietary histories. This calculation was proposed by Shivappa et al. [20] and modified by Tabung et al. [26]. The positive effects of pro-inflammatory nutrients such as vitamin B12, carbohydrate, cholesterol, energy, total fat, iron, protein, saturated fat and trans fat were added up, and then the negative effects of anti-inflammatory nutrients such as vitamin B6, fiber, folic acid, Mg, MUFA, niacin, omega-3 fatty acid, omega-6 fatty acid, PUFA, riboflavin, selenium, thiamin, vitamin A, vitamin C, vitamin D and vitamin E were subtracted. One limitation of our study is that we could not include all 45 components of the dietary inflammatory index that were proposed by Shivappa et al. [20]. This has also been the case in other studies, where the number of included dietary components ranged between 17 and 44 [21,22,26,32].

The components of dietary inflammatory index might also have an individual impact on weight regain. Therefore, correlation analyses were done separately based on each component. We showed that the intake of several micronutrients with anti-inflammatory properties, such as magnesium, riboflavin and folate was negatively correlated with weight regain. However, from those three micronutrients, only riboflavin intake remained significantly correlated with weight regain after correction for physical activity. Regular physical activity has been shown to have anti-inflammatory properties [33].

There are no prior data on the association between dietary magnesium, folate and riboflavin and the protection against weight regain in overweight and obese individuals. We suggest that this effect is due to the anti-inflammatory properties of

those micronutrients. Magnesium is suggested as a protective factor against oxidative stress, since hypomagnesemia was accompanied by a greater degree of oxidative stress in humans [34]. Folate has been associated with a reduction of inflammatory signals in overweight individuals [35]. Additionally, a low folate concentration was correlated with increasing risk of obesity [36]. These results suggest that there may be a potential role for magnesium and folate intake in body weight regulation via their effect on inflammation.

A role for riboflavin in the regulation of body weight has not yet been established or understood. It was previously shown that dietary riboflavin was protective against obesity [36]. In contrast, a population-based ecological study showed that a higher consumption of riboflavin per capita was associated with increased risk for obesity [37]. In vitro, induction of riboflavin in adipocytes was able to reduce pro-inflammatory factors such as tumor necrosis factor alpha, interleukin-6, MCDP-1 and HMGB1, while at the same time increasing anti-inflammatory markers such as adiponectin and interleukin 10. In addition, riboflavin supplementation in vitro was able to prevent macrophage infiltration in adipose tissue [38]. Riboflavin deficiency was associated with higher cellular stress in adipocytes, as shown by increased obesity-related apoptosis, reactive oxygen species and inflammation markers [39]. A high dietary intake of riboflavin might therefore be hypothesized to reduce weight loss-induced adipose tissue stress and inflammation and thus prevent weight regain.

This study has several strengths and limitations. To evaluate the association between dietary intake and weight regain after weight loss, this study applied an adequate study design with rigorous and strict weight loss regimes as well as a well-managed monitoring system. Compared to other long-term lifestyle-based weight loss studies, the drop-out rate of the subjects in this study was low. Limitations of this study include the small number of participants and the data collection on dietary intake for which we relied on three days of self-reported dietary records, which are known to be biased by underreporting. Underreporting of total energy intake and specific nutrients, such as saturated fat and sugar, will influence the calculation of the DII. However, this may be less important for the correlation analyses, if underreporting is comparable in all participants. Another limitation of the study is that the DII was formulated based on a limited number of items, and thus, the effect of other items in the DII will be underestimated in this study. The DII explained only ~9% of the variation in weight regain in our study. However, very strong associations are not to be expected given the complexity of body weight regulation. Several other factors, including a reduction in basal and activity-related energy expenditure, a higher level of physical activity, changes in hunger- and satiety-associated hormones, and an increase in fat cell stress in response to fat mass reduction have also been suggested to play a role [7,8,23]. Moreover, a causal relationship between DII and weight regain cannot be derived from this study. To confirm these results, further studies are needed to evaluate the effect of diets with lower inflammatory index on weight regain in an experimental design.

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In summary, we found no evidence for a role of macronutrient composition of diet after a weight loss intervention for the prevention of weight regain in this study. On the other hand, the inflammatory properties of the diet during the weight maintenance period may play a role in weight regain after a diet-induced weight loss program in overweight and obese adults. Further research should investigate whether tailoring diet with the aim to reduce the dietary inflammatory index is a potential approach to improve weight maintenance.

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Disclosure

The authors declare no conflict of interest.

Author Contribution

HFLM: Harry Freitag Luglio Muhammad; RGV : Roel G Vink; NJTR : Nadia JT Roumans; LAJA: Laura AJ Arkenbosch; ECM : Edwin C Mariman; MAVB : Marleen A van Baak; “MAVB and ECM conceived and designed the experiments; NJTR, LAJA, RGV performed the experiments; HFLM analyzed the data; HFLM, MAVB and ECM wrote the paper. All authors read and approved the manuscript”.

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Supplementary Table 3.1. Anthropometric data throughout the weight loss intervention.

	Baseline (M1)		Weight Loss Period (M2)		Weight Stable Period (M3)		Follow Up Period (M4)	
	LCD (n=29)	VLCD (n=28)	LCD (n=29)	VLCD (n=28)	LCD (n=29)	VLCD (n=28)	LCD (n=28)	VLCD (n=27)
Age (years)	51.8 ± 1.9	50.7 ± 1.5						
Height (cm)	171.7 ± 1.6	172.9 ± 1.8						
Weight (kg)	92.4 ± 1.9	92.6 ± 1.8	84.2 ± 1.9	83.6 ± 1.6	84.0 ± 1.9	83.3 ± 1.7	88.4 ± 2.0	88.1 ± 1.9
BMI (kg/m ²)	31.3 ± 0.5	31.0 ± 0.4	28.6 ± 0.5	28.0 ± 0.4	28.5 ± 0.5	27.9 ± 0.4	30.0 ± 0.5	29.2 ± 0.5
Fat mass (%)	39.9 ± 1.8	39.7 ± 1.5	34.5 ± 2.1	35.0 ± 1.8	33.8 ± 2.1	33.8 ± 1.8	36.5 ± 2.0	36.1 ± 1.8
Fat free mass (%)	60.1 ± 1.8	60.3 ± 1.5	65.5 ± 2.1	65.0 ± 1.8	66.2 ± 2.1	66.2 ± 1.8	63.5 ± 2.0	63.9 ± 1.8
Fat mass (kg)	36.6 ± 1.7	36.2 ± 1.2	29.0 ± 1.9	28.8 ± 1.4	28.3 ± 2.0	27.5 ± 1.3	32.2 ± 2.0	31.3 ± 1.5
Fat free mass (kg)	55.4 ± 2.2	55.9 ± 2.2	54.9 ± 2.1	54.3 ± 2.2	55.2 ± 2.2	55.1 ± 2.3	56.0 ± 2.3	56.1 ± 2.3
Waist circumference (cm)	102.6 ± 2.0	101.9 ± 1.5	95.3 ± 1.8	94.2 ± 1.4	94.3 ± 2.0	95.0 ± 1.3	98.3 ± 1.9	97.9 ± 1.6
Hip circumference (cm)	110.8 ± 1.3	111.1 ± 1.1	105.8 ± 1.4	105.2 ± 1.0	104.6 ± 1.4	104.9 ± 0.9	106.7 ± 1.8	105.7 ± 1.3

No significant differences were found between LCD and VLCD at any time point.

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Supplementary Table 3.2. Data on dietary intake, dietary inflammatory index, and physical activity at D2 and D3.

	Weight Stable (D2)			Follow Up (D3)		
	LCD	VLCD	All	LCD	VLCD	All
Energy intake (kcal/day)	1666 ± 89	1614 ± 91	1641 ± 64	1773 ± 147	1827 ± 100	1800 ± 88
Protein (%) ^a	21.9 ± 1.0	20.5 ± 0.8	21.2 ± 0.6	18.2 ± 1.0	19.4 ± 0.8	18.8 ± 0.6**
Carbohydrate (%) ^a	46.6 ± 1.6	47.2 ± 0.9	46.9 ± 0.9	48.5 ± 1.4	45.3 ± 1.6	46.9 ± 1.1
Sugars (%) ^a	22.1 ± 1.5	19.3 ± 1.2	20.8 ± 1.0	12.9 ± 1.1	11.06 ± 0.77	11.97 ± 0.69***
Fat (%) ^a	30.2 ± 1.3	31.0 ± 1.0	30.7 ± 0.8	30.9 ± 1.2	33.2 ± 1.5	32.1 ± 0.9
Saturated fat (g/ 1000 kcal) ^b	11.6 ± 0.6	11.9 ± 0.5	11.7 ± 0.4	11.9 ± 0.8	13.7 ± 0.8	12.8 ± 0.6
Trans fat (g/ 1000 kcal) ^b	0.87 ± 0.15	0.90 ± 0.07	0.89 ± 0.09	0.79 ± 0.09	1.00 ± 0.10	0.89 ± 0.068
MUFA (mg/ 1000 kcal) ^b	12.2 ± 0.8	11.7 ± 0.5	11.9 ± 0.5	11.9 ± 0.5	12.8 ± 0.61	12.3 ± 0.4
PUFA (mg/ 1000 kcal) ^b	6.36 ± 0.37	7.40 ± 0.45	6.85 ± 0.30	7.15 ± 0.45	6.66 ± 0.54	6.91 ± 0.35
Omega 3 fatty acids (mg/ 1000 kcal) ^b	0.88 ± 0.08	0.83 ± 0.07	0.86 ± 0.05	0.91 ± 0.14	0.85 ± 0.10	0.88 ± 0.08
Omega 6 fatty acids (mg/ 1000 kcal) ^b	4.98 ± 0.30	5.79 ± 0.44	5.36 ± 0.26	5.36 ± 0.37	5.27 ± 0.45	5.31 ± 0.29
Cholesterol (mg/ 1000 kcal) ^b	116.6 ± 12.1	96.5 ± 9.1	107.2 ± 7.8	98.72 ± 10.01	115.5 ± 12.9	107.1 ± 8.2
Fiber (g/ 1000 kcal) ^b	13.82 ± 0.70	15.09 ± 0.93	14.42 ± 0.57	12.79 ± 0.86	12.40 ± 0.59	12.59 ± 0.52 ^f
Alcohol (g/ 1000 kcal) ^b	1.93 ± 0.66	1.76 ± 0.75	1.85 ± 0.49	3.26 ± 0.92	3.02 ± 0.93	3.14 ± 0.65
Magnesium (mg/ 1000 kcal) ^b	194.9 ± 7.1	188.3 ± 7.1	191.8 ± 5.0	181.7 ± 10.7	177.9 ± 6.7	179.8 ± 6.2
Iron (mg/ 1000 kcal) ^b	7.17 ± 0.32	7.10 ± 0.26	7.14 ± 0.21	7.31 ± 0.55	6.89 ± 0.21	7.10 ± 0.29
Selenium (mg/ 1000 kcal) ^b	35.2 ± 2.6	28.6 ± 2.4	32.1 ± 1.8	28.3 ± 2.3	28.9 ± 2.8	28.6 ± 1.8
Zinc (mg/ 1000 kcal) ^b	6.19 ± 0.23	6.17 ± 0.36	6.18 ± 0.21	5.45 ± 0.43	5.56 ± 0.30	5.50 ± 0.26 ^e
Vitamin A (µg/ 1000 kcal) ^b	355.5 ± 29.6	626.0 ± 134.2	483.1 ± 67.2	403.2 ± 44.1	451.0 ± 58.3	427.1 ± 36.3
Vitamin D (mg/ 1000 kcal) ^b	1.88 ± 0.19	2.09 ± 0.18	1.98 ± 0.13	2.08 ± 0.27	2.19 ± 0.50	2.13 ± 0.28
Vitamin E (mg/ 1000 kcal) ^b	6.45 ± 0.45	6.52 ± 0.59	6.49 ± 0.36	7.17 ± 0.58	6.60 ± 0.60	6.89 ± 0.42
Thiamin (mg/ 1000 kcal) ^b	0.82 ± 0.05	0.80 ± 0.06	0.81 ± 0.04	0.85 ± 0.08	0.67 ± 0.05	0.76 ± 0.05
Riboflavin (mg/ 1000 kcal) ^b	1.06 ± 0.07	0.97 ± 0.07	1.02 ± 0.05	0.91 ± 0.09	0.88 ± 0.10	0.89 ± 0.07
Vitamin B6 (mg/ 1000 kcal) ^b	1.36 ± 0.09	1.41 ± 0.10	1.38 ± 0.07	1.25 ± 0.10	1.07 ± 0.08	1.16 ± 0.07**
Folate (µg/ 1000 kcal) ^b	129.4 ± 9.6	115.9 ± 9.7	123.03 ± 6.8	121.4 ± 15.19	106.6 ± 5.1	114.0 ± 7.99
Vitamin B12 (mg/ 1000 kcal) ^b	3.57 ± 0.77	3.06 ± 0.73	3.33 ± 0.53	2.94 ± 0.64	2.80 ± 0.34	2.86 ± 0.36
Niacin (mg/ 1000 kcal) ^b	11.92 ± 0.76	10.99 ± 0.67	11.48 ± 0.51	10.84 ± 0.92	10.30 ± 0.69	10.57 ± 0.57
Vitamin C (mg/ 1000 kcal) ^b	76.2 ± 8.1	57.2 ± 6.5	67.2 ± 5.4	52.5 ± 7.0	57.4 ± 6.3	54.9 ± 4.7
Dietary Inflammatory Index [^]	4.37 ± 0.36	4.10 ± 0.44	4.24 ± 0.28	4.60 ± 0.54	5.14 ± 0.35	4.87 ± 0.32
Physical Activity	9.21 ± 0.22	8.97 ± 0.18	9.06 ± 0.14	8.95 ± 0.20	8.60 ± 0.21	8.80 ± 0.14**

^aDietary intake as % of total energy intake; ^bDietary intake in gram or milligram per 1000 kcal of total energy intake; [^]Dietary inflammatory index: The sum of dietary inflammatory scores of each nutrient as calculated by Tabung et al [26]; P value * < 0.05, ** < 0.01 and *** < 0.001 for difference between D2 and D3 (paired t-test or Wilcoxon test). No significant differences were found between LCD and VLCD groups (independent t-test) at any time point. LCD: low calorie diet group ; VLCD: very low calorie diet group, MUFA: mono-unsaturated fatty acid; PUFA: poly-unsaturated fatty acid.



THE INTERACTION

BETWEEN INFLAMMATORY
PROPERTIES OF DIET AND
GENETIC VARIATION IN

BODY WEIGHT REGULATION

CHAPTER 4

Low Calorie Low Inflammatory Diet is Beneficial to Reduce hs-CRP during a Weight Loss Intervention among Individuals with Obesity

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ABSTRACT

Obesity is associated with increased systemic inflammation. The objective of this study was to investigate the effect of a low calorie low inflammatory diet (LCID) on inflammation markers, body weight and metabolic syndrome parameters in obese adults. We hypothesize that LCID can provide a beneficial effect on inflammation markers in addition to a reduction of body weight and metabolic syndrome parameters. This was a randomized controlled trial conducted for 8 weeks in 61 male and female Indonesian adults with obesity (51 completers). Subjects were randomly divided into 2 groups: a low-calorie diet (LCD) and a LCID group. Markers of inflammation (hs-CRP, TNF-alpha, and IL-6), anthropometric variables and metabolic syndrome parameters (blood pressure, lipid profile, and fasting glucose) were measured before and at the end of the intervention. At the end of the intervention, there was a significant increase of plasma TNF-alpha ($p < 0.001$) and IL-6 concentrations ($p = 0.015$), but no significant changes in hs-CRP level ($p = 0.707$) in the whole group. Subjects in the LCID group had a lower hs-CRP ($p = 0.029$), compared to those in the LCD group, but no difference was seen in TNF-alpha ($p = 0.319$) and IL-6 ($p = 0.628$). Subjects in both groups had a significant reduction in body weight, body fat, lipid profile and blood pressure (all $p < 0.05$) with no differences between groups (all $p > 0.05$). This study showed that the LCID had beneficial effects on hs-CRP during a weight loss intervention compared to the LCD but not on TNF-alpha, and IL-6, and this effect was independent of changes in body composition. Compared to LCD, LCID did not influence difference in the changes of body weight, body composition and metabolic syndrome parameters markers.

Keywords: inflammation, diet, obesity, weight loss, CRP.

INTRODUCTION

Obesity affects millions of adults worldwide and this condition is associated with increased risk for non-communicable diseases, such as type 2 diabetes mellitus and cardiovascular diseases [1]. Inflammation has been suggested as an important mediator between obesity and the development of cardiovascular diseases [2,3]. Obesity is associated with increased systemic and adipose tissue inflammation [4]. Hypertrophic adipose tissue shows elevated immune cell infiltration and higher production of pro-inflammatory cytokines [4]. The role of inflammation in the development of diabetes mellitus, hypertension, and dyslipidemia has been investigated in the past few decades [3] and studies suggest that inflammation leads to insulin resistance, disruption of the renin-angiotensin system, and damage of the arterial wall [3]. The insulin resistance then leads to increased production of hepatic lipoproteins leading to dyslipidemia.

In individuals with obesity, weight loss interventions are associated with a reduction of the clinical signature of cardiovascular diseases and diabetes mellitus (such as elevated blood pressure, triglycerides, low-density lipoprotein cholesterol, fasting glucose and HbA1c) [5] as well as of premature mortality [6]. Weight loss was also associated with a reduction in circulating inflammation markers, such as hs-CRP (high sensitivity C-reactive protein), in several studies [7-9]. However, other studies found no reduction in hs-CRP after weight loss [10,11] and adipose tissue inflammatory pathways have been reported to be up-regulated during calorie restriction and these pathways were only gradually down-regulated during the follow-up period [12,13]. In addition to hs-CRP, previous investigations showed a controversy whether other inflammatory markers such as plasma TNF-alpha and IL-6 concentrations were increased or unchanged at the end of a weight loss program [14,15,16].

One of the factors affecting the inflammatory status is diet composition [17]. Several tools to assess the inflammatory properties of the diet have been developed such as the dietary inflammatory index (DII) [18], the dietary inflammation score [19] and the food-based index of dietary inflammatory potential [20]. The score on these indices has been shown to be associated with systemic inflammation in population-based studies [19-22].

The main objective of this study was to investigate the effect of weight loss on different inflammatory markers and whether this effect is modifiable by diet in adults with obesity. Thus, we developed a Low Calorie Low Inflammatory Diet (LCID), a diet with the aim to induce a calorie deficit as well as to reduce inflammation during a weight loss program. The diet was inspired by Mediterranean diet principle which previously was shown to reduce inflammation [23]. We hypothesize that LCID can provide a beneficial effect on inflammation markers in addition to a reduction of body weight and metabolic syndrome parameters. If the composition of the energy-restricted diet is indeed able to have a (further) beneficial effect on the inflammatory status, this might lead to an improved standard for dietary counseling for weight loss.

MATERIALS AND METHODS

Study Subjects

This was a randomized controlled trial in Indonesian male and female obese adults. The intervention was conducted in Yogyakarta, Indonesia. The inclusion criteria for this study were adults between 21 and 56 years old and body mass index (BMI) higher than 27.5 kg/m². The cut off point is lower in Asian population because the health risk is greater than those non-Asian population with the same BMI [24]. The exclusion criteria were current chronic diseases such as type 2 diabetes mellitus and heart diseases, elevated blood pressure (systolic > 140 mm Hg and/or diastolic > 90 mmHg) and blood glucose concentrations (higher than 11.1 mmol/L for random and 6.94 mmol/L for fasting glucose), pregnancy, breastfeeding, smoking, long-term consumption of certain drugs (such as metformin, allopurinol and simvastatin), being on a weight loss diet or using weight loss supplements, drugs or herbal preparations. This study followed the ethical guidelines of the 1975 Declaration of Helsinki. Registered under Medical and Health Research Ethics Committee, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Indonesia identifier number KE/0560/05/2017.

Subjects were recruited by announcements via flyers, a webpage, and social media in October 2017. A total of 195 individuals applied to become participants via short messages, phone, and email. Of those 195, 77 subjects had a BMI above 27.5 kg/m². Those subjects were then invited to the university for measurements of blood glucose and blood pressure. 61 subjects were eligible and willing to participate in the study after having the protocol explained, including the changes in dietary intake and an exercise routine at the university 2 times a week. The minimum number of subjects in each group was calculated based on β value 10%, α value 0.05, expected mean difference in hs-CRP = 1.3 mg/L and standard deviation = 1.9 mg/L (moderate change) [25]. Result from the calculation was a total of 23 subjects in each group.

Eligible subjects were randomized into 2 groups, the low-calorie diet group (LCD) and the low calorie low inflammatory diet group (LCID). The randomization was done using an online randomization tool (<https://www.randomizer.org>) stratified for sex. Subjects were blinded to the type of dietary group while researchers and nutritionists were not blinded. The intervention included dietary counseling and an exercise program. The duration of the intervention was 8 weeks.

Dietary Recommendations

The dietary recommendations for weight loss in this study were given by trained nutritionists. The counseling sessions were done at the beginning of the study, and every 2 weeks thereafter. Subjects were asked to stay on the LCD and LCID regime until post-test assessments were done.

The dietary recommendations given by nutritionists were based on two components: 1) explanation of the principles of the diet and 2) prescribing a one-day meal plan that could be followed by the participant. Subjects were allowed to change their meal plan by exchange with other meals with similar caloric and macronutrient contents. They were provided with a list of foods and their portion size with a similar calorie and macronutrient content. If subjects had problems following the meal plan that had been provided, the nutritionists could help them arrange the closest meal plan that the subject could achieve. The recommended one-day meal plan was divided into 5 meals per day: breakfast, lunch, dinner and 2 snacks (morning and afternoon).

Subjects in this study were divided into two groups: LCD and LCID. The recommendation for total energy intake was the same in both groups, i.e. 1500 kcal per day. Subjects in both groups also had the same distribution of calories over their daily meals. However, subjects in the LCD and the LCID group followed different dietary principles. Subjects in the LCD group only had to adhere to the reduction in energy intake, subjects in the LCID group were also given advice on how to lower the inflammatory properties of their diet. For instance, consuming white meat or soy products instead of red meat as the source of protein; always eat fruit in between meals as snacks, avoid sugary food such as cake and other party products. Details about the difference in dietary advice between the low-calorie diet and low calorie low inflammatory diet groups can be found in Table 4.1.

Table 4.1. The dietary recommendations in the LCD and LCID groups.

Low-Calorie Diet (LCD) (1500 kcal/day)	Low Calorie Low Inflammatory Diet (LCID) (1500 kcal/day)
<ul style="list-style-type: none"> • Eat and drink according to the prescribed diet. • Always read the nutritional facts before eating any packaged food or check the energy content. • Distribute your food over 5 meals per day • When consuming milk, please make sure that it is preferably skimmed or semi-skimmed and the yogurts skimmed and without added sugar. • Consume fresh fruits, preferably seasonal fruit • Avoid fruit juice as it loses its fibre but retains its sugar. • Avoid consumption of fatty sausages. • Drink preferably drinking water or infusions without sugar. • Avoid the consumption of sweetened, carbonated and alcoholic beverages. 	<ul style="list-style-type: none"> • Same recommendations as LCD group plus: • Use olive oil as main culinary fat. • Consume a minimum of 2 servings of vegetables per day. • Consume a minimum of 3 servings of fruits per day. • Increase consumption of legumes (3 serving per day). • Increase consumption of nuts (3 serving per day). • Increase consumption of fish or shellfish (1-3 servings per day). • Change meat products from red meat (beef, goat and lamb) into chicken or fish. • Avoid consuming sweets and pastries • Avoid consuming butter, margarine and cream • Use seasoning like garlic, onion, ginger for cooking • Drink tea, coffee or ginger with no or limited sugar.

The LCID regime was based on the principles of the Mediterranean diet (<https://dietamediterranea.com/nutricion-saludable-ejercicio-fisico/>) because this type of diet was previously shown to be associated with a reduction of inflammatory markers compared with a typical western diet [23]. The compliance with this dietary recommendation was assessed by the Mediterranean Diet score [26] as well as from the intake of its food components (vegetables, fruits, white meat, red meat, legumes and fish/seafood).

Exercise

The exercise was an integral part of this intervention study. Subjects in the LCD and LCID groups followed the same exercise regime. This included one session of aerobic exercise and one session of dumbbell exercise every week. The dumbbell combined-resistance exercise was a combination of aerobic and strength exercise where subjects used dumbbells or their own weight. The duration of each exercise session was 60 minutes, which was composed of 15 minutes of warming-up, 30 minutes of core exercise and 15 minutes of cooling down. All the exercise sessions were done in a group setting with an instructor leading the group exercises. In total, subjects undertook 16 sessions of exercise. The exercise program was the same in both groups. Subjects in the LCD and LCID groups exercised in different group sessions with a different schedule. The aim of this separation was to prevent information exchange between subjects in the two groups.

Anthropometric Measurements

Anthropometric measurements were done to evaluate the effect of diet on changes in adiposity. The anthropometric measures included body weight (kg), body mass index (BMI) (kg/m^2), total body fat (%), visceral fat (%), subcutaneous fat (%), muscle mass (%), waist circumference (cm), hip circumference (cm), and waist-to-hip ratio (WHR). Body weight, percent body fat, total body fat, visceral fat, subcutaneous fat, and muscle mass were measured using a bioelectrical impedance body composition monitor and scale (0.01 kg precision; Omron Karada Scan HBF-375, Osaka, Japan). Height was measured using a wall-mounted tape (0.1 cm precision, GEA medical, Jakarta, Indonesia). Waist and hip circumference were measured using a non-elastic tape (0.1 cm precision). All anthropometric measurements were done by trained personnel using calibrated instruments at baseline and at the end of the intervention.

Assessment of dietary intake and Mediterranean Diet (MD) score

Dietary intake was assessed before the intervention (week 0) and at the end of the intervention (week 9). The dietary intake at week 0 and week 9 was assessed using a semi-quantitative food frequency questionnaire (SQ-FFQ). Together with the SQ-FFQ, a separate questionnaire was used to assess the Mediterranean Diet (MD)

score [26]. The SQ-FFQ that was used in this study has been validated [27] and used before in the same location in a similar study population [28]. The analysis of the SQ-FFQ data was based on the food data base of Indonesian foods and that of the United States Department of Agriculture [29]. Data collection for the SQ-FFQ was done by a face-to-face interview with trained nutritionists. The SQ-FFQ contains data on the amount of foods consumed and the frequency. Data on foods intake were transformed into the amount of consumed foods per day. Furthermore, analysis of individual nutrient intakes was done for each food and then compiled into total daily nutrient intake. The intake of energy, protein, fat, saturated fat, trans fat, cholesterol, monounsaturated fatty acid, polyunsaturated fatty acid, omega 3 fatty acid, omega 6 fatty acid, carbohydrate, fibre, alcohol, iron, magnesium, selenium, zinc, vitamin A, thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, vitamin C, vitamin D, vitamin E, beta carotene, tea and caffeine was estimated because their association with inflammation [18].

The MD score was assessed according to Martínez-González et al [26]. The score was calculated based on 14 questions regarding foods and drinks that are encouraged or avoided in a Mediterranean diet: 1) The usage of olive oil as main culinary fat; 2) daily consumption of olive oil; 3) daily consumption of vegetables; 4) daily consumption of fruit; 5) daily consumption of red meat and other meat products; 6) daily consumption of butter, margarine or cream; 7) daily consumption of carbonated beverages; 8) weekly consumption of wine; 9) weekly consumption of legumes; 10) weekly consumption of fish or seafood; 11) weekly consumption of commercial sweets and pastries; 12) weekly consumption of nuts; 13) preferred consumption of white meat instead of red meat; 14) weekly consumption of sofrito (tomato sauce). A higher MD score represents higher adherence towards the Mediterranean diet principle.

Physical activity was assessed using the global physical activity questionnaire (GPAQ) [30]. This questionnaire contains information on the intensity and duration of several activities including work/job, transportation, house-related work and maintenance, recreation, exercise, and leisure-time physical activity. Each activity has a unique MET (metabolic equivalent of task) score, which represents the amount of energy used for a certain type of activity as multiple of resting metabolic rate. In order to obtain an overall picture of the individual's physical activity, all activities that have been reported in GPAQ are transformed into MET-minutes/week. The GPAQ was developed, validated for the Indonesian population and used before [30].

Assessment of markers of the metabolic syndrome

Blood pressure was measured using an Omron HEM 7120 Automatic Blood Pressure device (Omron, Japan). This measurement was done after at least 10 minutes rest from recent activity and the subjects were asked to sit in a comfortable sitting position with their left arm fully exposed and resting on a supportive surface at heart level.

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Blood pressures were measured three times on the left arm using an appropriate cuff size. For analysis, the average value of those three measurements was used.

Plasma concentrations of metabolic and inflammation markers were measured in blood samples which were collected in the morning after an 8-10 hours fast. A 5 mL blood sample was collected in a ethylenediaminetetraacetic acid (EDTA)-containing tube. After collection, blood plasma was separated by centrifugation and stored at -80oC. Plasma concentrations of total cholesterol, high density lipoprotein cholesterol (HDL-c) and low-density lipoprotein cholesterol (LDL-c) were measured using the CHOD-PAP enzymatic photometric test. Plasma triglyceride concentration was measured using the glycerol phosphate oxidase (GPO) method. Fasting plasma glucose concentration was measured using the glucose hexokinase method (Diasys, Holzheim, Germany).

The presence of metabolic syndrome was established at baseline and at the end of the intervention based on the International Diabetes Federation guideline [31]. The parameter of metabolic syndrome includes central obesity (marked by waist circumference higher than 90 cm for men or 80 cm for women) and at least two of the following components: 1) raised triglycerides (higher than 1.7 mmol/L); 2) reduced HDL-c (lower than 1.03 mmol/L for men or 1.29 mmol/L for women); 3) raised blood pressure (systolic higher than 130 mmHg or diastolic higher than 85 mmHg); 4) raised fasting glucose (higher than 5.6 mmol/L). Metabolic syndrome score was calculated by adding one of those components (waist circumference, triglyceride, HDL-C, blood pressure and fasting glucose) that higher than cut off value in sex-specific manner.

Assessment of inflammation markers

Fasting plasma concentrations of hs-CRP, TNF-alpha and IL-6 were measured at baseline and at the end of the weight loss intervention. Hs-CRP, TNF-alpha and IL-6 were measured using enzyme immunoassays (DRG, USA and FineTest, China).

Statistical Analysis

JASP software (Version 0.8.3.1, The University of Amsterdam) was used for statistical analysis in this study. The anthropometric measures, metabolic syndrome components, inflammation markers, dietary intake, and physical activity were compared between subjects in the LCD and LCID group at baseline using an independent t-test. The difference in dietary intake (including total energy, nutrients intake, and Mediterranean diet score) between LCD and LCID, before and during intervention were analyzed using repeated measure ANOVA. The analysis was done on those with a complete dataset for each measurement. Main outcomes were the intervention (time) effect, the group effect and the time x group interaction. Similar

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to dietary intake, the anthropometric measures, metabolic syndrome parameters and inflammation markers were compared between LCD and LCID group, before and at the end of the intervention using repeated measure ANOVA. Adjustment for sex and body fat changes was applied to the analysis of metabolic syndrome parameters, and inflammation markers. The relationships between dietary components during a weight loss program and inflammatory markers in the total group of subjects were examined using linear regression analysis. Statistical significance was obtained when the p-value was less than 0.05. All statistical analyses were conducted 2-tailed.

RESULTS

This was a randomized controlled trial conducted among obese male and female adults in Indonesia. From 61 subjects who initially participated in this study, 30 subjects were assigned to the LCD group 31 subjects were assigned to the LCID group. Ten subjects dropped out (5 subjects in each group) during the intervention with the following reasons: 1) unable to follow the dietary regime and exercise due to personal reasons, 2) loss of interest and 3) loss of contact (Figure 4.1). There were no significant differences in anthropometric measures, metabolic syndrome parameters or inflammatory markers between the LCD and LCID group (Table 4.2; all $p > 0.05$). Additionally, subjects in both groups had a comparable energy intake, and physical activity (Table 4.2; all $p > 0.05$).

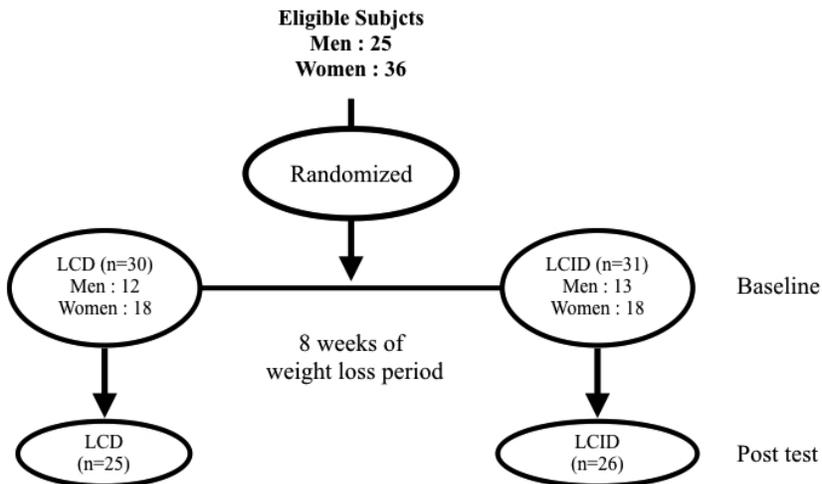


Figure 4.1. Study flow diagram.

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Table 4.2. Characteristics of subjects at baseline.

	LCD (n=30)	LCID (n=31)	p*
Male/female	12/18	13/18	
Age (years)	24.7 ± 5.3	24.0 ± 5.9	0.613
Anthropometric measures			
Height (cm)	161.6 ± 10.1	161.6 ± 9.6	0.994
Weight (kg)	87.4 ± 13.5	87.3 ± 16.8	0.984
BMI (kg/m ²)	33.5 ± 4.1	33.1 ± 4.9	0.731
Body fat (%)	35.9 ± 4.9	35.6 ± 5.6	0.826
Visceral fat (%)	16.2 ± 16.2	15.5 ± 6.1	0.644
Subcutaneous fat (%)	31.6 ± 7.9	31.1 ± 8.6	0.815
Muscle (%)	25.3 ± 3.7	25.5 ± 4.1	0.843
Waist circumference (cm)	96.5 ± 9.8	97.5 ± 10.7	0.711
Hip circumference (cm)	110.1 ± 7.5	109.3 ± 10.5	0.730
Waist-to-hip ratio	0.88 ± 0.07	0.90 ± 0.06	0.233
Energy intake (kcal/day)	2281 ± 936	2158 ± 908	0.605
Dietary inflammatory index score	5.3 ± 4.4	5.0 ± 4.4	0.789
Physical activity (METs-min/weeks)	1273 ± 2946	1845 ± 2385	0.407
Markers of the metabolic syndrome			
Glucose (mmol/L)	5.69 ± 0.96	5.74 ± 1.3	0.897
Triglycerides (mmol/L)	1.47 ± 0.71	1.16 ± 0.55	0.066
Total cholesterol (mmol/L)	4.85 ± 0.92	4.93 ± 0.82	0.713
HDL-c (mmol/L)	1.71 ± 0.31	1.70 ± 0.23	0.857
LDL-c (mmol/L)	4.60 ± 1.06	4.70 ± 0.74	0.655
Systolic blood pressure (mmHg)	112.5 ± 11.6	114.7 ± 9.7	0.422
Diastolic blood pressure (mmHg)	79.9 ± 8.3	80.9 ± 7.1	0.614
Inflammation markers			
hs-CRP (mg/L)	7.0 ± 4.1	6.6 ± 3.1	0.642
TNF-alpha (pg/mL)	10.8 ± 3.4	11.1 ± 3.2	0.702
IL-6 (pg/mL)	2.2 ± 1.6	3.5 ± 2.8	0.068

*Independent t-test. Data are presented as mean ± standard deviation. BMI: body mass index; hs-CRP: high sensitive C-reactive protein; HDL-c: high density lipoprotein cholesterol; IL-6: interleukin 6; LDL-c: low density lipoprotein cholesterol; TNF-alpha: tumor necrosis factor alpha; LCD: low calorie diet; LCID: low calorie low inflammatory diet

Dietary intake and physical activity of 47 out of 51 subjects were recorded before and at the end of the weight loss intervention (Table 4.3). Subjects in both groups reduced their dietary intake and there was no significant difference in total energy intake between groups ($p=0.901$). The adherence to the low-calorie diet was not very strict in both groups, thus only a modest reduction in body weight was reported. We

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found no significant time x group interactions for macro and micronutrient intakes nor for physical activity during the intervention (all $p > 0.05$). The MD score in both groups as well as intakes of MD score components (diet compositions) are shown in Supplementary Table 4.1. Subjects in the LCD as well as the LCD group had a small increment in the MD score ($p = 0.034$) during the intervention and no differences were seen between groups ($p = 0.645$). There was no difference in physical activity, including participation in the exercise program, between the LCD and LCD groups during the weight loss program ($p = 0.585$).

Table 4.3. Dietary intake and physical activity before and during the weight loss program.

	Pre		Post		p^*	p^\wedge	p^+
	LCD (n=23)	LCID (n=24)	LCD (n=23)	LCID (n=24)			
Energy (kcal)	2233 ± 203	2143 ± 200	1946 ± 130	1817 ± 146	0.053	0.565	0.901
Protein (g/1000kcal)	32.9 ± 1.7	32.2 ± 1.6	30.5 ± 1.5	33.3 ± 1.4	0.668	0.542	0.221
Fat (g/1000kcal)	30.5 ± 2.3	28.4 ± 2.2	26.1 ± 1.7	27.5 ± 1.8	0.135	0.868	0.331
Saturated fat (g/1000kcal)	12.6 ± 1.0	11.8 ± 1.0	10.5 ± 0.7	11.2 ± 0.8	0.143	0.978	0.417
Trans fat (g/1000kcal)	0.10 ± 0.02	0.09 ± 0.02	0.06 ± 0.02	0.09 ± 0.03	0.088	0.661	0.088
Cholesterol (mg/1000kcal)	104.9 ± 12.8	96.4 ± 9.3	87.8 ± 11.8	116.7 ± 12.5	0.881	0.428	0.078
MUFA (g/1000kcal)	10.2 ± 0.8	9.5 ± 0.7	8.9 ± 0.7	9.4 ± 0.7	0.254	0.938	0.335
PUFA (g/1000kcal)	5.1 ± 0.4	5.0 ± 0.4	4.4 ± 0.4	4.5 ± 0.3	0.057	0.917	0.641
Omega 3 fatty acid (g/1000kcal)	0.14 ± 0.02	0.11 ± 0.02	0.14 ± 0.02	0.11 ± 0.02	0.995	0.245	0.774
Omega 6 fatty acid (g/1000kcal)	0.40 ± 0.07	0.38 ± 0.05	0.30 ± 0.05	0.41 ± 0.05	0.485	0.418	0.228
Carbohydrate (g/1000kcal)	146.5 ± 5.7	149.6 ± 5.7	159.8 ± 3.9	151.9 ± 4.1	0.073	0.656	0.203
Fiber (g/1000kcal)	8.2 ± 0.4	8.9 ± 0.6	9.8 ± 0.8	9.7 ± 0.6	0.026	0.611	0.394
Alcohol (g/1000kcal)	0.0 ± 0.0	0.6 ± 0.6	0.05 ± 0.04	0.01 ± 0.01	0.385	0.369	0.302
Fe (mg/1000kcal)	6.9 ± 0.3	6.8 ± 0.4	6.0 ± 0.3	6.7 ± 0.4	0.080	0.499	0.232
Mg (mg/1000kcal)	130.0 ± 8.1	141.0 ± 10.0	135.6 ± 11.9	141.4 ± 10.4	0.704	0.492	0.762
Se (µg/1000kcal)	43.1 ± 1.5	40.9 ± 1.3	41.0 ± 1.0	43.3 ± 1.6	0.880	0.957	0.060
Zn (mg/1000kcal)	4.0 ± 0.2	3.9 ± 0.1	3.8 ± 0.2	3.9 ± 0.1	0.556	0.994	0.344
Vitamin A (RE/1000kcal)	242.6 ± 29.5	275.1 ± 39.2	254.3 ± 32.5	384.5 ± 55.0	0.109	0.071	0.194
Thiamin (mg/1000kcal)	0.52 ± 0.02	0.52 ± 0.02	0.54 ± 0.02	0.53 ± 0.02	0.312	0.816	0.983
Riboflavin (mg/1000kcal)	0.54 ± 0.02	0.52 ± 0.02	0.50 ± 0.04	0.61 ± 0.04	0.756	0.202	0.158
Niacin (mg/1000kcal)	8.2 ± 0.5	8.0 ± 0.4	8.0 ± 0.4	8.9 ± 0.5	0.430	0.474	0.195
Vitamin B6 (mg/1000kcal)	0.62 ± 0.04	0.63 ± 0.04	0.67 ± 0.06	0.75 ± 0.05	0.067	0.412	0.382
Folate (µg/1000kcal)	126.6 ± 12.9	144.5 ± 19.8	130.3 ± 18.5	155.4 ± 16.6	0.596	0.289	0.785
Vitamin B12 (µg/1000kcal)	1.1 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	0.886	0.844	0.480
Vitamin C (mg/1000kcal)	71.8 ± 14.2	77.5 ± 11.0	82.2 ± 17.1	84.4 ± 12.1	0.475	0.792	0.893
Vitamin D (µg/1000kcal)	0.8 ± 0.2	0.7 ± 0.2	0.9 ± 0.2	0.8 ± 0.1	0.151	0.547	0.480
Vitamin E (mg/1000kcal)	1.1 ± 0.2	1.2 ± 0.2	1.4 ± 0.3	1.3 ± 0.2	0.364	0.984	0.804
Beta carotene (µg/1000kcal)	1391.8 ± 211.7	1695.3 ± 323.5	1551.3 ± 249.5	2355.9 ± 373.4	0.113	0.108	0.325
Tea (g/1000kcal)	0.90 ± 0.20	0.56 ± 0.10	0.59 ± 0.16	0.57 ± 0.13	0.137	0.308	0.202
Caffeine (g/1000kcal)	0.030 ± 0.010	0.004 ± 0.004	0.004 ± 0.004	0.013 ± 0.007	0.001	0.056	0.056
Physical activity (METs-min)	1579 ± 688	2002 ± 516	1831 ± 321	1724 ± 248	0.978	0.735	0.585

Data are presented as mean ± standard error of mean; METs-min: metabolic equivalent of tasks-minute; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; LCD: low calorie diet; LCID: low calorie low inflammatory diet; Analysis were done using repeated-measure ANOVA *within subjects effects, ^between subjects effects, +time x group interaction.

At the end of the intervention, there was a significant increase of plasma TNF-alpha ($p<0.001$) and IL-6 concentrations ($p=0.015$), but no significant changes in hs-CRP level ($p=0.707$) in the whole group. After correction for sex and changes in body fat, subjects in the LCID group had a greater reduction in hs-CRP concentration than those in the LCD group ($p=0.029$) (Figure 4.2). There were no significant differences in changes of TNF-alpha ($p=0.319$) and IL-6 ($p=0.628$) between the LCD and LCID groups (Figure 4.2). We also found a significant reduction in body weight, body mass index, percent body fat, triglyceride, cholesterol, HDL-cholesterol and diastolic blood pressure, and an increase in muscle mass percentage in the total group (all $p<0.050$), but no differences between the diet groups (Table 4.4).

Table 4.4. Changes in anthropometric and metabolic syndrome parameters.

Variables	Pre		Post		p^*	p^\wedge	p^\dagger	$p^\#$
	LCD (n=25)	LCID (n=26)	LCD (n=25)	LCID (n=26)				
Anthropometric measures								
Weight (kg)	86.6 ± 2.8	85.0 ± 2.9	85.0 ± 2.7	83.9 ± 3.0	<0.01	0.740	0.416	N/A
BMI (kg/m ²)	33.3 ± 0.8	33.0 ± 1.1	32.7 ± 0.9	32.5 ± 1.2	<0.01	0.860	0.902	N/A
Body fat (%)	36.0 ± 1.1	36.0 ± 1.0	35.1 ± 1.2	35.4 ± 1.1	<0.01	0.929	0.459	N/A
Visceral fat (%)	15.8 ± 1.1	15.3 ± 1.3	14.8 ± 1.1	14.8 ± 1.3	<0.01	0.913	0.186	N/A
Subcutaneous fat (%)	32.0 ± 1.7	31.4 ± 1.7	31.2 ± 1.8	30.9 ± 1.7	<0.01	0.849	0.283	N/A
Muscle (%)	25.2 ± 0.8	25.3 ± 0.8	25.5 ± 0.9	25.4 ± 0.8	0.049	0.990	0.627	N/A
Waist circumference (cm)	96.5 ± 2.0	95.9 ± 2.1	96.1 ± 1.5	94.2 ± 2.4	0.298	0.653	0.517	N/A
Hip circumference (cm)	110.2 ± 1.4	108.4 ± 2.1	107.9 ± 1.7	107.7 ± 2.0	0.076	0.174	0.345	N/A
Waist-to-hip ratio	0.89 ± 0.01	0.90 ± 0.01	0.90 ± 0.02	0.87 ± 0.02	0.504	0.511	0.206	N/A
Metabolic syndrome parameters								
Glucose (mmol/L)	5.7 ± 0.2	5.87 ± 0.3	5.9 ± 0.2	6.0 ± 0.2	0.120	0.770	0.992	0.917
Triglycerides (mmol/L)	1.4 ± 0.1	1.1 ± 0.1	1.2 ± 0.2	1.0 ± 0.1	0.046	0.159	0.773	0.705
Cholesterol (mmol/L)	4.9 ± 0.2	4.9 ± 0.2	4.4 ± 0.2	4.6 ± 0.2	<0.01	0.616	0.311	0.594
HDL-c (mmol/L)	1.7 ± 0.1	1.7 ± 0.1	1.4 ± 0.0	1.6 ± 0.1	<0.01	0.301	0.147	0.309
LDL-c (mmol/L)	4.6 ± 0.2	4.7 ± 0.1	4.4 ± 0.2	4.4 ± 0.2	0.261	0.776	0.995	0.716
Systolic bp (mmHg)	113.0 ± 2.5	112.8 ± 2.0	109.5 ± 2.7	110.1 ± 2.7	0.081	0.943	0.825	0.665
Diastolic bp (mmHg)	80.0 ± 1.8	80.7 ± 1.4	77.5 ± 1.9	76.5 ± 1.7	0.008	0.933	0.509	0.609
Metabolic syndrome score	1.9 ± 0.2	1.8 ± 0.2	2.2 ± 0.2	1.8 ± 0.2	0.226	0.249	0.226	0.169

Data are presented as mean ± standard error of mean; repeated measure ANOVA for *within subjects effects, ^between subjects effects, †group x time interaction; #group x time interaction with correction for sex and changes in body fat. BMI: body mass index; hs-CRP: high sensitive C-reactive protein; HDL-c: high density lipoprotein cholesterol; IL-6: interleukin 6; LDL-c: low density lipoprotein cholesterol; TNF-alpha: tumor necrosis factor alpha.

To further evaluate the influence of the inflammatory properties of the diet on changes in inflammatory markers, linear regression analyses were done with correction for sex and changes in body fat. As shown in Supplementary Table 4.2, in all subjects of both groups intakes of protein, selenium, zinc, niacin, beta carotene and cholesterol were negatively correlated with changes in hs-CRP (all $p<0.05$). None

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of the nutrient intakes were correlated with changes of TNF-alpha. By contrast, intake of tea ($p=0.019$) and caffeine ($p=0.028$) were negatively correlated with changes in IL-6.

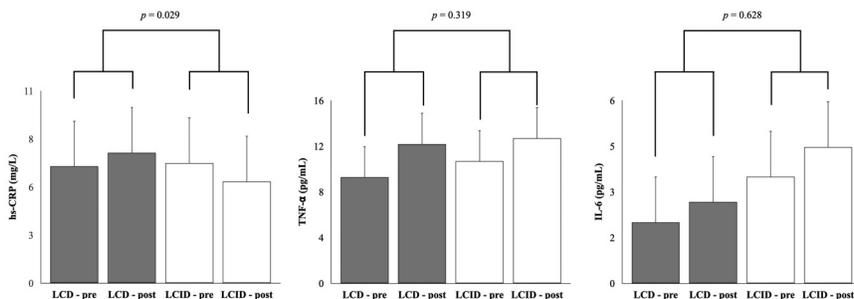


Figure 4.2. Inflammatory markers before and after intervention separated by intervention group. Data are presented as mean (standard deviation). Analysis was done using repeated measure ANOVA with correction for sex and changes in body fat. There was a significant increase of plasma TNF-alpha ($p<0.001$) and IL-6 concentrations ($p=0.015$), but no significant changes in hs-CRP level ($p=0.707$) in the whole group. The p value in the figure reflected differences in changes between dietary groups. LCD: low calorie diet; LCID: low calorie low inflammatory diet; hs-CRP: high sensitivity C-reactive protein; TNF-alpha: tumor necrosis factor-alpha; IL-6: interleukin 6.

DISCUSSION

The general aim of this study was to investigate the effect of weight loss on inflammatory markers and to evaluate whether changes in systemic inflammation can be modified by diet. The hypothesis of this study was partly accepted. At the end of the intervention, we found increased plasma TNF-alpha and IL-6 concentrations, but no change in hs-CRP concentration in the total group. Interestingly, when comparing the diet effect, we showed that LCID group had a greater reduction in hs-CRP at the end of the intervention compared to those in the LCD group. No differences were seen in changes of TNF-alpha and IL-6 concentrations between groups. The weight loss intervention in this study induced significant reductions in body weight, body fat, and metabolic syndrome parameters such as plasma triglycerides and blood pressure, with no differences between groups.

In the scientific literature reports on the effects of weight loss on the inflammatory status of individuals with obesity are inconsistent. Some investigators found a reduction in hs-CRP after a weight loss intervention [7,8], others report unchanged hs-CRP concentrations [10,11] and unchanged [14] or increased plasma TNF-alpha and IL-6 concentrations [15,16] have also been measured. In this study, we showed that a weight loss program using a low-calorie diet and exercise program was associated with an increase in plasma TNF-alpha and IL-6, but no change in hs-CRP concentrations. Differences among studies may be due to the type of weight

loss intervention (level of energy restriction, duration and total weight loss, with or without exercise). The finding that not all inflammatory markers respond in the same way is not surprising. hs-CRP is mainly derived from the liver, whereas adipose tissue more likely contributes to the changes in TNF-alpha and IL-6 concentration. Although all subjects participated in an exercise training program during the weight loss intervention, it does not seem very likely that the exercise bouts contributed to the increased plasma levels of IL-6, because the increased release of IL-6 by the exercising skeletal muscles is quickly normalized after stopping the exercise [32]. The weight loss-induced increase in plasma TNF-alpha and IL-6 is in line with other reports showing that inflammatory gene expression pathways are upregulated in adipose tissue during a weight loss program [12,13,33].

Another factor that may be responsible for the inconsistent findings with respect to weight loss-induced changes in inflammatory markers may be the diet composition. In our study, we showed that the hs-CRP concentration was on average not affected by the weight loss program in the total group. However, when the group was divided based on type of diet, we showed that hs-CRP was lower in the LCD group compared to the LCD group. No group difference was found for the changes in TNF-alpha and IL-6. It can be speculated that the weight loss-induced increase in inflammatory status of the adipose tissue is not sensitive to changes in the inflammatory properties of the diet, whereas the liver remains responsive to the diet composition under the conditions of energy restriction. However, more evidence should be obtained to further support this suggestion.

To our knowledge, this is the first study to evaluate the effect of dietary counseling with a combination of an attempt to lower energy intake as well as to lower inflammatory properties of the diet during a weight loss intervention. The notion to reduce inflammation by means of a dietary intervention has been applied in studies in Australia (AUSMED Heart Trial) [34,35] and in the United States (IMAGINE study) [36]. In comparison to other studies which aimed to lower inflammation through modification of diet, the strength of our study was that all other factors such as gender distribution, age, recommended energy intake, and training program were controlled by design which leaves only the effect of lowering the inflammatory property of the diet as the independent variable.

The analysis of inflammation markers was done at the end of the weight loss program whilst subjects were still in the energy deficit state. It was previously reported by Vink et al [13] that during a weight-loss period, there was an increase in gene expressions related to inflammation followed by a reduction in those gene expressions during a weight stable period. Thus, it is argued that because the timing of this analysis was done at the end of the weight-loss period with participants still in negative energy balance, subjects had increased inflammation markers. This is marked by higher TNF-alpha and IL-6 than the baseline.

A limitation of this study was that it could not show significant differences in changes in dietary intake between the LCID and LCD groups. This might be due to inadequacy of SQ-FFQ and MD score to capture small differences in dietary intake in combination with the small sample size. Not all principles of the Mediterranean diet, such as increased consumption of dairy, potatoes, olives, and wine, could be applied in the Indonesian population. Differences in dietary intake between the groups may have been small, because the intake of several components that contribute to the MD score were already high or low at baseline in this population. For instance, consumption of wine is low-to-none and because alcohol consumption is prohibited among Moslems it will not increase during the intervention. Additionally, consumption of red meat was already low at baseline among subjects, because legumes (tempe or tofu) and chicken meat were the preferred sources of protein, leaving little room for a further reduction. Some subjects experienced difficulty in complying with the diet on a daily basis because of several factors including appetite, work/job, social events, food availability and economic condition. The Indonesian population usually consumes rice as the source of carbohydrates instead of potatoes, pasta and bread. Indonesians are also less likely to consume dairy products such as milk and cheese, especially during adulthood. Nevertheless our results showed a difference in response of hs-CRP with weight loss between the groups. Replication of this result in other populations is required before an anti-inflammatory weight loss diet can be propagated.

In summary, we found that reducing the inflammatory properties of the diet during a weight loss program can help to reduce systemic inflammation, as marked by hs-CRP level, in adults with obesity. On the other hand, such a diet does not prevent the increment of adipose tissue inflammation, as marked by TNF-alpha and IL-6 concentrations, due to energy restriction. This may suggest that the liver is responsive to diet composition during a period of energy restriction, whereas adipose tissue is not. To confirm this finding, follow-up studies should be done.

Conflict of Interest Statement

There is no financial and personal relationship with other people or organizations that could inappropriately influence this work.

Statement of Authorship

Conceptualization, H.F.L.M., M.A.v.B. and E.C.M, Methodology, H.F.L.M., M.A.v.B. and E.C.M, Investigation, H.F.L.M., Resources, H.F.L.M. Data Curation, H.F.L.M., M.A.v.B. and E.C.M.; Writing – Original Draft Preparation, H.F.L.M., M.A.v.B. and E.C.M.; Writing – Review & Editing, H.F.L.M., M.A.v.B. and E.C.M.; Supervision, M.A.v.B. and E.C.M; Project Administration, H.F.L.M.; Funding Acquisition, H.F.L.M. Harry Freitag Luglio Muhammad (H.F.L.M.), Marleen A. van Baak (M.A.v.B.) and Edwin C. Mariman (E.C.M.)

Trial Registration

Registered under Medical and Health Research Ethics Committee, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Indonesia identifier number KE/0560/05/2017, website : <http://komisietik.fk.ugm.ac.id>

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Supplementary Table 4.1. The Mediterranean Diet score and daily intake of food components

	Pre		Post		p*	p^	p+
	LCD (n=23)	LCID (n=24)	LCD (n=23)	LCID (n=24)			
Mediterranean diet score	5.2 ± 1.8	5.4 ± 1.2	5.8 ± 1.4	6.1 ± 1.6	0.034	0.440	0.654
Vegetables (g/day)	126.0 ± 18.1	118.1 ± 14.4	121.8 ± 13.3	145.6 ± 22.9	0.447	0.790	0.210
Fruits (g/day)	136.3 ± 22.1	188.6 ± 36.2	202.7 ± 31.6	247.7 ± 38.6	0.024	0.206	0.863
Legumes (g/day)	112.5 ± 15.0	96.7 ± 12.7	56.1 ± 6.6	65.8 ± 14.7	0.001	0.771	0.263
Fish and seafood (g/day)	16.3 ± 2.8	14.7 ± 3.5	22.2 ± 7.6	15.1 ± 3.1	0.535	0.353	0.585
White meat (g/day)	38.1 ± 11.0	32.6 ± 4.0	32.6 ± 4.8	44.3 ± 7.6	0.670	0.720	0.165
Red meat (g/day)	21.8 ± 3.9	11.8 ± 2.5	13.7 ± 3.9	8.5 ± 1.7	0.021	0.054	0.461

Data are presented as mean ± standard deviation; LCD: low calorie diet; LCID: low calorie low inflammatory diet; repeated measure ANOVA for *within subject effects, ^between subjects effects, +group x time interaction.

Supplementary Table 4.2. Correlation between dietary components during weight loss program on changes in inflammation markers.

Dietary intake during weight loss intervention	Changes of hs-CRP				Changes of TNF-alpha				Changes of IL-6			
	B*	P	B^	P	B*	P	B^	P	B*	P	B^	P
Energy (kcal)	0.090	0.546	0.111	0.523	-0.276	0.067	-0.091	0.590	0.068	0.671	0.075	0.683
Protein (g/1000kcal)	-0.296	0.043	-0.363	0.030	0.193	0.203	0.012	0.941	-0.151	0.345	-0.104	0.555
Fat (g/1000kcal)	-0.003	0.982	0.001	0.993	0.142	0.351	0.121	0.422	-0.012	0.939	-0.010	0.955
Carbohydrate (g/1000kcal)	0.065	0.663	0.083	0.599	-0.091	0.553	-0.028	0.855	0.093	0.561	0.074	0.664
Fiber (g/1000kcal)	-0.076	0.610	-0.046	0.802	0.158	0.300	-0.002	0.989	-0.061	0.703	-0.064	0.743
Fe (mg/1000kcal)	-0.227	0.124	-0.233	0.183	0.164	0.283	0.090	0.609	-0.109	0.498	-0.126	0.487
Mg (mg/1000kcal)	-0.214	0.148	-0.226	0.215	0.110	0.471	-0.023	0.989	-0.141	0.378	-0.182	0.328
Se (µg/1000kcal)	-0.399	0.005	-0.435	0.006	-0.026	0.865	-0.093	0.553	0.214	0.179	0.290	0.104
Zn (mg/1000kcal)	-0.241	0.102	-0.332	0.048	0.235	0.120	0.045	0.786	-0.128	0.426	-0.045	0.806
Vitamin A (RE/1000kcal)	-0.229	0.121	-0.259	0.117	0.044	0.773	-0.060	0.710	-0.050	0.758	-0.021	0.905
Thiamin (mg/1000kcal)	-0.025	0.868	-0.006	0.974	0.210	0.165	0.122	0.473	-0.143	0.374	-0.166	0.375
Riboflavin (mg/1000kcal)	-0.305	0.037	-0.326	0.054	0.035	0.818	-0.087	0.601	-0.217	0.174	-0.242	0.163
Niacin (mg/1000kcal)	-0.316	0.031	-0.378	0.020	0.152	0.319	0.034	0.833	0.072	0.654	0.158	0.381
Vitamin B6 (mg/1000kcal)	-0.210	0.157	-0.280	0.119	0.215	0.155	0.030	0.865	-0.097	0.548	-0.057	0.770
Folate (µg/1000kcal)	-0.291	0.047	-0.322	0.071	0.095	0.533	-0.020	0.910	-0.103	0.521	-0.138	0.463
Vitamin B12 (µg/1000kcal)	-0.297	0.043	-0.300	0.073	-0.060	0.696	-0.134	0.414	-0.120	0.455	-0.120	0.492
Vitamin C (mg/1000kcal)	-0.164	0.270	-0.152	0.375	0.156	0.305	0.023	0.890	-0.116	0.472	-0.119	0.507
Vitamin D (µg/1000kcal)	-0.283	0.054	-0.268	0.114	0.001	0.996	-0.024	0.884	-0.044	0.786	-0.096	0.617

CHAPTER 4

Low Calorie Low Inflammatory Diet is Beneficial to Reduce hs-CRP during a Weight Loss Intervention among Individuals with Obesity

Dietary intake during weight loss intervention	Changes of hs-CRP				Changes of TNF-alpha				Changes of IL-6			
	B*	P	B^	P	B*	P	B^	P	B*	P	B^	P
Vitamin E (mg/1000kcal)	-0.252	0.088	-0.254	0.158	0.032	0.833	-0.067	0.703	-0.035	0.830	-0.073	0.705
Saturated fat (g/1000kcal)	0.068	0.650	0.053	0.737	0.083	0.588	0.084	0.580	0.068	0.671	0.082	0.647
MUFA (g/1000kcal)	-0.010	0.948	0.002	0.990	0.134	0.381	0.145	0.337	0.032	0.845	0.023	0.891
PUFA (g/1000kcal)	-0.070	0.639	-0.020	0.905	0.107	0.484	0.115	0.472	-0.128	0.425	-0.171	0.320
Trans fat (g/1000kcal)	-0.169	0.255	-0.196	0.211	0.058	0.707	-0.058	0.703	-0.013	0.936	0.048	0.780
Cholesterol (mg/1000kcal)	-0.468	<0.001	-0.602	<0.001	0.050	0.743	0.001	0.994	0.104	0.517	0.127	0.539
Omega 3 fatty acid (g/1000kcal)	-0.277	0.059	-0.270	0.099	-0.070	0.647	-0.149	0.349	-0.080	0.619	-0.052	0.766
Omega 6 fatty acid (g/1000kcal)	-0.132	0.375	-0.224	0.163	0.165	0.279	-0.117	0.451	-0.042	0.792	0.076	0.655
Alcohol (g/1000kcal)	0.042	0.780	-0.082	0.608	0.297	0.048	-0.158	0.302	-0.209	0.190	-0.105	0.541
Tea (g/1000kcal)	-0.089	0.550	-0.051	0.745	-0.062	0.685	0.045	0.772	-0.337	0.031	-0.383	0.019
Caffeine (g/1000kcal)	-0.083	0.580	-0.062	0.694	-0.080	0.600	0.070	0.647	-0.338	0.031	-0.360	0.028
Beta carotene (µg/1000kcal)	-0.249	0.091	-0.348	0.032	0.123	0.421	-0.090	0.573	-0.044	0.784	0.001	0.995

Linear regression test *without correction and ^with correction for sex and changes in body fat, B= standardized coefficient, p= significance level.

CHAPTER 5

The Effect of a Low Calorie Low Inflammatory Diet on Relative Telomere Length and Mitochondrial DNA Copy Number among Obese Indonesian Adults

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ABSTRACT

We developed a low calorie low inflammatory diet (LCID) which has been previously reported to reduce the pro-inflammatory marker high sensitive C-reactive protein (hs-CRP). It was previously reported by other study that higher inflammation is associated with reduction of mitochondrial DNA copy number (mtDNA-CN) and relative telomere length (RTL) which in turn could increase susceptibility of degenerative diseases. The objective of this study was to evaluate the effect of LCID on mtDNA-CN and RTL. This is a randomized controlled trial among Indonesian adults with obesity. Participants were divided into 2 groups: low-calorie diet (LCD) and low calorie low inflammatory diet (LCID). Participants in both groups undertook 2 times a week a supervised exercise program. The length of the weight loss program was 8 weeks, and participants were followed for 8 weeks of the weight maintenance period. 61 participants initially started the program, 51 finished the weight loss period, and 35 finished the maintenance period. The mtDNA-CN and RTL were evaluated at baseline and after the weight loss program. The mtDNA-CN was unchanged ($p=0.960$) after weight loss while RTL was shown to be increased ($p=0.002$). Changes in RTL were higher in LCID than those in the LCD group ($p=0.036$), while mtDNA-CN showed no difference. Weight changes after weight loss and maintenance period were not associated with mtDNA-CN or RTL (all $p>0.05$). We concluded that a weight loss program was associated with the elongation of telomere length among adults with obesity. Those in LCID responded with significantly longer telomeres as compared to LCD despite a similar degree of weight loss.

Keywords: telomere, mitochondrial DNA, obesity, weight loss, inflammation.

introduction

Obesity affects millions of adults worldwide and this state of over-nutrition is associated with increased risk for non-communicable diseases (NCDs) [1,2]. One of the mechanisms, which may explain the interaction between obesity and the development of NCDs, is inflammation [3-7]. Inflammation plays a role in the regulation of several molecular signatures such as mitochondrial function and telomere length. Increased inflammation is associated with the reduction of mitochondrial DNA copy number (mtDNA-CN), an indicator for mitochondrial biogenesis and function [8]. Evidence of mitochondrial involvement in the pathogenesis of obesity through mitochondrial dysfunctions leading to oxidative stress has been reported [9]. In addition, increased inflammation is also associated with the shortening of telomere length. In humans, concentrations of inflammatory proteins such as high-sensitive C-reactive protein (hs-CRP), interleukin 6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha) were reported to be negatively associated with relative telomere length (RTL) [10,11]. Telomere shortening may predispose individuals to age-related diseases and increased mortality [12].

Studies in the past decades suggested that mtDNA-CN and RTL are molecular markers for aging [13]. Interestingly, it also has been shown that mtDNA-CN and RTL are related to an individual's body weight. A recent prospective study reported that reductions in mtDNA-CN and RTL were associated with weight gain [14]. This finding is supported by cross-sectional studies which show an inverse correlation between mtDNA-CN and adiposity in Korean [15] and Italian populations [16]. The RTL was also inversely associated with adiposity measures such as body mass index and waist circumference in a large epidemiological study in the United States [12]. The tendency of a negative correlation between obesity and RTL was then confirmed by a meta-analysis of data from 39 studies [17].

MtDNA-CN and RTL were previously reported to be affected by lifestyle factors, such as diet and physical activity. Among individuals with overweight, intervention with a low-fructose and low-sodium diet for 8 weeks was associated with an increment of mtDNA-CN [18]. In patients with chronic kidney disease, conducting a resistance exercise program for 12 weeks was associated with an improvement of mtDNA-CN [19]. The role of dietary components in regulating RTL has been reported in epidemiological studies. Consumption of sugar-sweetened beverages and meat was associated with the shortening of RTL while consumption of antioxidant-rich plants was associated with RTL maintenance [20].

From the literature, it can be concluded that there were association between mtDNA-CN, RTL and systemic inflammation due to increased adiposity. It was previously reported that mtDNA-CN and RTL are associated with weight gain, and those parameters are influenced by diet and physical activity. However, the majority of reports on the role of dietary components on changes in mtDNA-CN and RTL among

adults are based on observational studies. Therefore, conducting an intervention study is important to evaluate whether diet and exercise had an influence on mtDNA-CN and RTL.

METHODS

Participants and study group

This is a secondary analysis of a clinical trial conducted among Indonesian men and women with obesity. The intervention was conducted in Yogyakarta, Indonesia. The inclusion criteria in this trial (Chapter 4) were adults between 21 and 56 years old and body mass index (BMI) higher than 27.5 kg/m². The exclusion criteria were current chronic diseases such as type 2 diabetes mellitus and heart diseases, elevated blood pressure and blood glucose concentrations, pregnancy, breastfeeding, smoking, long-term consumption of certain drugs (such as metformin, allopurinol and simvastatin), being on a weight loss diet or using weight loss supplements, drugs or herbal formula. Ethical clearance was obtained from the Medical and Health Research Ethics Committee, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Indonesia (KE/0560/05/2017). This study followed the ethical guidelines of the 1975 Declaration of Helsinki.

Participants were recruited by announcements via fliers, a webpage, and social media. A total of 195 individuals applied to become participants via short messages, phone, and email. Of those 195, 77 participants had a BMI above 27.5 kg/m². Those participants were then invited to the university (Universitas Gadjah Mada, Yogyakarta, Indonesia) for measurements of blood glucose and blood pressure. Those with high blood glucose (fasting higher than 6.94 mmol/L or random higher than 11.1 mmol/L) or elevated blood pressure (higher than 140/90 mmHg) were not included in the study. 61 participants were eligible and willing to participate in the study after being informed of the study protocol, including the changes in dietary intake and an exercise routine at the university 2 times a week.

After stratification for sex, the participants were randomized (<https://www.randomizer.org>) into 2 groups, the low-calorie diet group (LCD) and the low calorie low inflammatory diet group (LCID). The intervention in this study included dietary counseling and an exercise program. The exercise consisted of 15 minutes warming-up, 30 minutes core exercise and 15 minutes cool down. A combination of aerobic and dumbbell exercises with low-to-medium exercise intensity and an estimated total calorie expenditure of 300 kcal was used. The exercise program was the same in both groups. The duration of the intervention was 8 weeks (Figure 5.1). Details about the weight loss program were published elsewhere (Chapter 4).

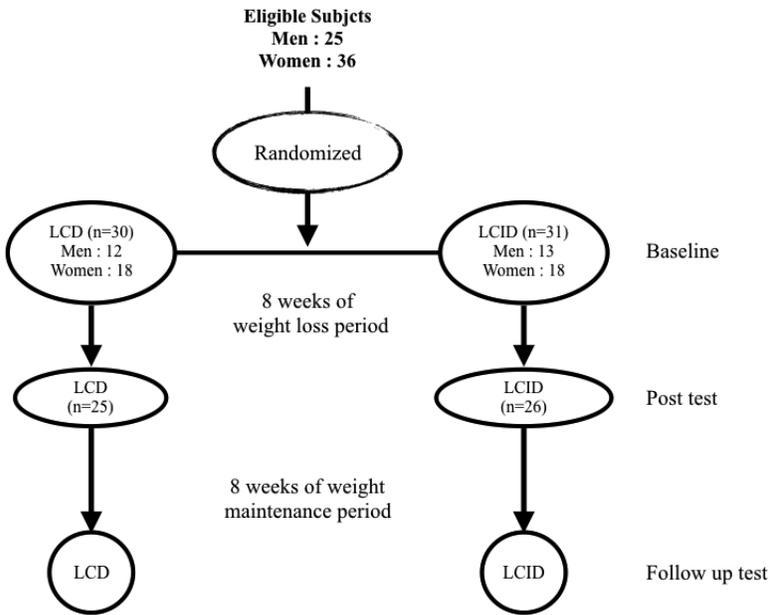


Figure 5.1. Study design

The dietary counseling was done to reduce energy intake and participants in both groups had similar 1500 kcal energy intake a day. While those in LCD only focused on reducing calorie intake, those with LCID had additional dietary recommendations to reduce inflammation. This is achieved by adapting Mediterranean diet principles to the low-calorie diet regime [21]. In our initial publication, we showed that LCD and LCID had a similar weight loss but those in the LCID group had a greater reduction in hs-CRP (Chapter 4).

Measurements

Measurements (anthropometrics and blood sampling) were performed at baseline, after the weight-loss period (week 9), and after the weight maintenance period (week 16). In this study, the anthropometric measures included body weight (kg) and body mass index (BMI) (kg/m²). Body weight was measured using a bioelectrical impedance body composition monitor and scale (0.01 kg precision; Omron, Osaka, Japan). Height was measured using a wall-mounted tape measure (0.1 cm precision, GEA medical, Jakarta, Indonesia). BMI was calculated by dividing body weight with squared height (in m). WC was measured at the midpoint between the lower margin of the least palpable rib and the top of the iliac crest using a non-elastic tape (0.1 cm precision).

Blood samples were collected in the morning after an 8-10 hours fast. From each subject, a 5 mL blood sample was collected in ethylenediaminetetraacetic

acid (EDTA)-containing tubes. After collection, blood plasma and buffy coat were separated by centrifugation and stored at -80°C. Genomic DNA was extracted from peripheral blood mononuclear cells (PBMC) using FavorPrep Blood Genomic DNA Extraction Mini Kit (Favorgen, Taiwan) for quantification of MtDNA-CN and RTL. The inflammation markers hs-CRP, leptin, TNF-alpha, and IL-6 were measured in plasma using enzyme-linked immunosorbent assay according to the manufacturer's protocol (DRG, New Jersey, USA; Finetest, Wuhan, China).

Quantification of MtDNA-CN and RTL

MtDNA-CN and RTL were determined by quantitative real-time PCR (qPCR) assay, using SYBR Select MasterMix detection (Applied Biosystem, Foster City, USA) in the 7500 Real-Time PCR System (Applied Biosystem), as previously described [22]. Briefly, the mtDNA-CN measurement was done by using primers for mitochondrial tRNA^{Leu} (*MT-TL1*) and nuclear-encoded β -2 microglobulin (*B2M*) genes, following the protocol from Venegas, *et al.* [23], while for telomere repeats, the measurement was conducted following the protocol by O'Callaghan *et al.* [24]. Copy number of *MT-TL1* gene and telomere repeats were normalized by the single copy *B2M* as a reference gene. MtDNA-CN and RTL were calculated based on the efficiency-corrected method as implemented in the "qPCR" package in R-Project for Statistical Computing version 3.4.3 (www.r-project.org), according to the five-parameter sigmoidal models fitted to the raw fluorescence data [25,26].

Statistical analyses

Statistical analysis was done using JASP software version 0.8.3.1 (University of Amsterdam). Changes of mtDNA-CN and RTL over the weight-loss period within groups were analyzed using the Wilcoxon signed-rank test. The between groups differences between mtDNA-CN and RTL at baseline and their changes with weight loss were evaluated using the Mann Whitney test and the ANCOVA test. The ANCOVA test was also used to evaluate the difference in changes in anthropometric measures, inflammation markers, mtDNA-CN, and RTL between weight gainers and weight losers. The analysis was done on those with a complete dataset for each measurement. The correlation of mtDNA-CN and RTL with body weight and inflammation markers was analyzed using the Spearman correlation test and linear regression test with correction for age, sex, and changes in body weight (for non-anthropometric analysis). A P-value of less than 0.05 was considered as statistically significant. This analysis was done in whole group and in the separate intervention groups (LCID vs LCD).

RESULTS

Details about changes in dietary intake, inflammation markers, and anthropometric measures have been reported elsewhere (Chapter 4). Of 51 participants who finished the weight loss program, 35 participants were followed after 8 weeks (during weight maintenance) (Figure 5.2). We found no differences in body weight changes between LCD and LCID during the weight-loss period ($p=0.443$) and during the weight maintenance period ($p=0.634$).

We also evaluated the differences in changes of mtDNA-CN and RTL during the different dietary interventions, LCID and LCD. The changes in mtDNA-CN and RTL in both groups are shown in Figure 5.3. There was no difference in mtDNA-CN and RTL at baseline between LCD and LCID ($p=0.154$, $p=0.605$, respectively). We found a significant increment of RTL ($p<0.001$), but no changes in mtDNA-CN ($p=0.641$) in the total group with weight loss program. When comparing between-group changes using an ANCOVA test with correction for age, sex, height and initial weight (Table 5.2), the result showed that those in the LCID group had a higher increment in RTL as compared to those in the LCD group ($p=0.036$), but no difference was seen in mtDNA-CN ($p=0.336$).

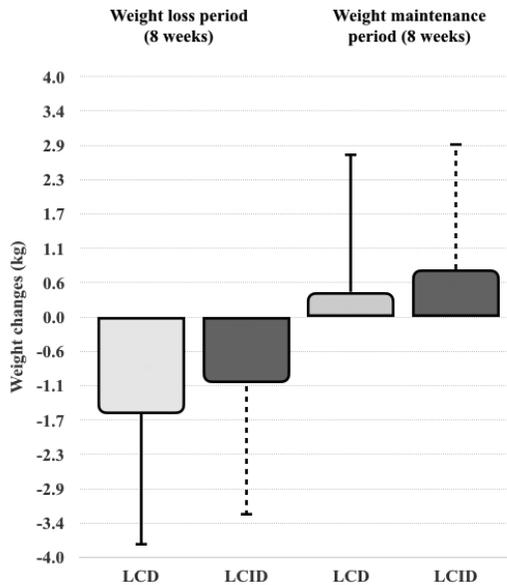


Figure 5.2. Body weight changes during weight loss period and during weight maintenance period. Data were presented as mean (standard deviation).

CHAPTER 5

The Effect of a Low Calorie Low Inflammatory Diet on Relative Telomere Length and Mitochondrial DNA Copy Number among Obese Indonesian Adults

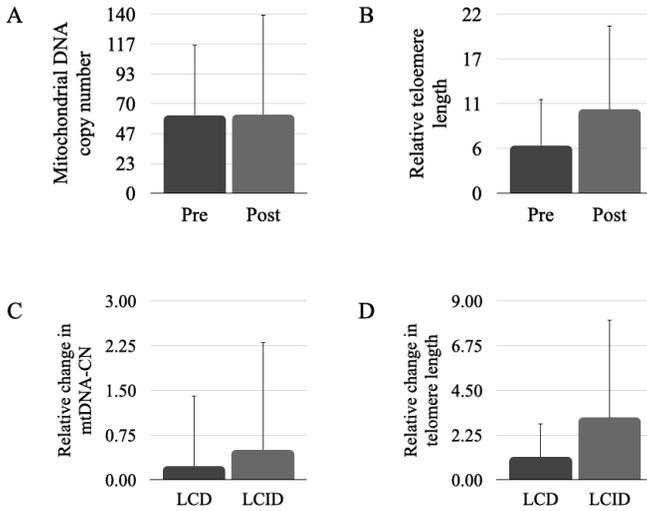


Figure 5.3. The mtDNA-CN and RTL before and after the weight loss period (A and B, respectively). The difference in relative changes of mtDNA-CN and RTL between LCD and LCID (C and D, respectively).

The correlations between mtDNA-CN and RTL with inflammation markers were analyzed at baseline and after the weight loss program. At baseline mtDNA-CN and RTL showed negative correlations with leptin and IL-6 (all $p < 0.05$) in the total group of participants, but no correlations with hs-CRP and TNF-alpha (Table 5.1). In separate group analyses, only those in the LCID group showed the negative correlations, consistent with the total group of participants.

Table 5.1. Correlation of inflammation markers with mitochondrial DNA copy number and relative telomere length at baseline.

	Mitochondrial DNA copy number		Relative telomere length	
	All (n=47)		All (n=47)	
Baseline values	R*	p	R*	p
hs-CRP (mg/L)	-0.113	0.456	-0.126	0.404
TNF-alpha (pg/mL)	-0.018	0.909	0.020	0.894
Leptin (ng/mL)	-0.416	0.009	-0.330	0.043
IL-6 (pg/mL)	-0.352	0.030	-0.495	0.002
Body weight (kg)	0.011	0.943	-0.061	0.685

*Spearman correlation test.

The correlation between changes in inflammation markers and changes in mtDNA-CN and RTL was also analyzed (Table 5.2). Among all participants, we found that changes in TNF-alpha were negatively correlated with percent changes

CHAPTER 5

of mtDNA-CN ($p=0.013$) while changes in other inflammation markers were not correlated with percent changes of mtDNA-CN (all $p>0.05$). We found that changes in RTL were not correlated with any inflammation marker in this study (hs-CRP, leptin, TNF-alpha and IL-6, $p>0.05$). To evaluate the influence of dietary intervention, further analysis was done by analyzing the correlation between changes in mtDNA-CN and RTL and changes in inflammation markers in the dietary groups separately. Among participants in the LCID group, the change in mtDNA-CN was negatively correlated with changes of TNF-alpha ($p=0.014$) and IL-6 ($p=0.053$, borderline significant) after correction for age, sex, and body weight change. In this group, the change of RTL was negatively correlated with change in TNF-alpha ($p=0.029$), but the association was no longer evident after correction for confounding factors. By contrast, among participants in the LCD group the change in IL-6 was positively correlated with the change in mtDNA-CN ($p=0.026$). In this group, the change in RTL was not correlated with changes of inflammation markers (all $p>0.050$).

Table 5.2. Correlation between changes in inflammation markers with relative changes in mtDNA-CN and RTL (n=47).

Correlation with mtDNA-CN change	All participants (n=47)				LCD (n=23)				LCID (n=24)			
	R	p	B	p	R	p	B	p	R	p	B	p
Change hs-CRP (mg/L)	0.053	0.724	-0.040*	0.799	0.158	0.469	-0.174*	0.465	0.021	0.921	-0.035*	0.884
TNF-alpha (pg/mL)	-0.101	0.507	-0.466*	0.013	0.238	0.287	-0.201*	0.545	-0.387	0.068	-0.615*	0.014
Leptin (ng/mL)	0.212	0.208	0.236*	0.272	-0.040	0.876	-0.221*	0.405	0.359	0.131	0.324*	0.398
IL-6 (pg/mL)	0.130	0.431	-0.028*	0.872	0.469	0.043	0.525*	0.026	-0.215	0.362	-0.463*	0.053
Weight loss (kg)	0.049	0.750	-0.048^	0.761	-0.053	0.821	-0.303^	0.216	0.137	0.533	0.094^	0.675

Correlation with RTL	All participants (n=47)				LCD (n=23)				LCID (n=24)			
	R	p	B	p	R	p	B	p	R	p	B	p
hs-CRP (mg/L)	-0.003	0.982	-0.143*	0.368	0.223	0.304	0.252*	0.296	-0.103	0.629	-0.241*	0.315
TNF-alpha (pg/mL)	-0.118	0.439	-0.103*	0.608	0.294	0.185	-0.276*	0.413	-0.455	0.029	-0.065*	0.817
Leptin (ng/mL)	0.064	0.706	0.340*	0.113	0.015	0.954	-0.303*	0.253	0.029	0.906	0.455*	0.239
IL-6 (pg/mL)	-0.081	0.623	-0.111*	0.521	0.082	0.739	0.038*	0.885	-0.280	0.232	-0.255*	0.323
Weight loss (kg)	-0.032	0.835	0.117^	0.467	-0.064	0.782	-0.152^	0.536	-0.013	0.954	0.166^	0.469

Linear regression analysis with *correction for age, sex and body weight changes or ^correction for age and sex; B : standardized value for linear regression test with correction for confounding factors; R: Spearman test; p : significant value with 2-tailed analysis.

Participants were separated based on their weight loser/gainer status (Table 5.3). Weight gainers and weight losers were defined by differences in weight changes from baseline (week 0) to weight maintenance period (week 16). Participants were weight losers when the net weight difference was negative while participants were weight gainer when net weight difference was 0 or positive. In this study, we showed that weight losers had higher body height ($p=0.045$), greater weight loss ($p<0.001$), and greater TNF-alpha increment ($p<0.001$) during the weight-loss period. In addition, we showed no difference in changes of mtDNA-CN ($p=0.587$) and RTL ($p=0.194$) between weight gainer and weight loser.

Table 5.3. Differences in anthropometric, inflammation, mtDNA-CN and RTL changes during weight loss between those who gained weight and those who lose weight after 8 weeks of follow up.

	Weight loser (n= 19)		Weight gainer (n=16)		p*	p^
	Mean	SD	Mean	SD		
Age (years)	25.1	5.4	24.1	5.1	0.468	-
Height (cm)	163.3	8.8	156.9	9.7	0.045	-
Anthropometric changes during weight loss						
Body weight (kg)	-3.02	1.94	-0.21	1.53	< 0.001	<0.001
Body fat (%)	-1.06	1.52	-0.43	0.61	0.163	0.547
Vis Fat (%)	-1.31	0.94	-0.27	0.65	0.002	0.009
BMI (kg/m ²)	-1.13	0.70	-0.07	0.61	< 0.001	<0.001
Subcutaneous fat (%)	-1.11	0.92	-0.34	0.54	0.012	0.044
Muscle (%)	0.42	0.72	0.23	0.30	0.468	0.916
Waist circumference (cm)	-0.36	5.97	0.17	6.53	0.914	0.285
Hip circumference (cm)	-4.73	6.14	0.22	3.92	0.023	0.090
WHR	0.04	0.07	0.00	0.08	0.301	0.752
Inflammation changes during weight loss						
hs-CRP (mg/L)	-0.06	2.97	-0.00	2.40	0.987	0.940
TNF-alpha (pg/mL)	3.54	1.67	1.05	2.15	0.002	<0.001
Leptin (ng/mL)	-4.28	3.71	-0.72	5.56	0.044	0.051
IL-6 (pg/mL)	1.24	1.93	0.40	1.44	0.589	0.259
mtDNA-CN and RTL changes during weight loss						
mtDNA-CN (relative changes)	0.20	1.28	0.43	1.40	0.343	0.587
RTL (relative changes)	0.99	1.73	1.95	3.20	0.325	0.194

*Mann Withney test; ^ANCOVA test with correction for age, sex, height and initial body weight. This analysis compares characteristic differences between weight losers and weight gainers. Participants were weight loser when the net weight difference from baseline to follow-up was negative while participants were weight gainer when the net weight difference was 0 or positive. MtDNA-CN: mitochondrial DNA copy number; RTL: relative telomere length.

DISCUSSIONS

Participants in both diet groups (LCD and LCID) had an increase in RTL but not in mtDNA-CN after the weight loss program. The increment in RTL was higher among participants in the LCID group than in the LCD group. However, changes in RTL were not associated with changes in any of the pro-inflammatory markers analyzed, while changes in mtDNA-CN were negatively correlated with changes of TNF-alpha. There was no association between changes in mtDNA-CN and RTL and weight regain status.

To our knowledge, there are few studies investigating the effect of a weight loss program on telomere length [27]. A study in adolescents with obesity showed that a weight loss program with modification of diet and physical activity was associated with elongation of telomere length [28]. The effect of energy restriction among adults was conflicting, where one study showed a lengthening effect in adult men with obesity [29], but no effect was seen in postmenopausal women with overweight/obesity [30]. By contrast, a correlation between weight loss and increasing telomere length was reported among adults after intragastric balloon therapy [31].

In this study, we showed an effect of a low-calorie diet on the elongation of telomere length in men and women with obesity, and by reducing the inflammation properties of the diet we were able to optimize the effect on telomere length. The reduction of the inflammatory properties of diet was obtained by adapting the principle of the Mediterranean diet in combination with the low-calorie diet prescription. It was previously reported that adherence to the Mediterranean diet principle was positively associated with longer telomere length [32]. The effect of Mediterranean diet principles on telomere length might be due to the combined effect of bioactive nutrients and phytochemicals contained in the recommended foods [33]. The effect of reducing inflammation on RTL in the LCID group is expected and in line with other studies. To our knowledge, this study is the first intervention study to evaluate the influence of Mediterranean diet principles in combination with a low-calorie diet on changes in telomere length. The majority of studies on the interaction between the Mediterranean diet and telomere length was based on an observational study [33].

Different from telomere length, this study showed that the weight loss program was not associated with changes in mtDNA-CN, and there were no differences in changes in mtDNA-CN between the LCD and LCID groups. As it was previously reported that mtDNA-CN was negatively associated with adiposity [14-16], we expected that weight loss in this study could increase mtDNA-CN. However, a weight loss program among individuals with obesity through diet or gastric banding was also not associated with changes of adipocyte mtDNA-CN [34]. It was suggested that other aspects of mitochondrial examination such as structure, function or size might provide a better insight on mitochondrial health rather than the mtDNA-CN [34,35].

Findings from this study support the notion that inflammation is inversely associated with mtDNA-CN and RTL. At baseline IL-6 and leptin were negatively correlated RTL. However, this study could not provide evidence that changes in RTL were associated with changes in the pro-inflammatory markers analyzed (hs-CRP, TNF-alpha or IL-6). The RTL improvement might be influenced by other inflammation markers such as IL-2 [36], which were not analysed in this study. In addition, this study was conducted in a relatively small number of subjects which make it hard to reach a significant value. Several mechanisms have been proposed on the interaction between inflammation and telomere length. First, inflammation is associated with a reduction of telomerase expression, the enzyme that is involved in the lengthening of telomeres. Second, inflammation also directly induces telomeric DNA damage mediated by reactive oxygen species [37].

Similar to RTL, IL-6 and leptin were inversely correlated with mtDNA-CN at baseline. Additionally, we showed that an increase in TNF-alpha after the intervention was associated with a reduction of mtDNA-CN. These findings suggest that more inflammation is associated with a reduction of mtDNA-CN among individuals with obesity. However, because of the nature of this study, we cannot draw conclusions about a causal relationship between inflammation and mtDNA-CN.

There are several limitations to this study. Because of the limitation of specimen collection due to ethical reasons, the mtDNA-CN and RTL in this study were analyzed from circulating leukocytes and not from adipose tissue, which would probably be more relevant in a weight loss study. It was previously reported that the association between inflammation, mtDNA-CN and RTL varied between tissues [38,39]. It was also reported that inflammation was positively correlated with adipocyte mtDNA-CN but negatively with monocyte mtDNA-CN [38]. Additionally, RTL from leukocytes is only significantly correlated with muscle RTL, but not with RTL of other tissues [39]. Another limitation in this study is that the number of participants was rather limited, thus further study with larger sample size is necessary to confirm the findings.

In conclusion, this study reported the effect of a weight loss intervention on changes in mtDNA-CN and RTL in PBMCs. We showed that reducing calorie intake and increasing physical activity among individuals with obesity was beneficial to increase RTL but had no effect on mtDNA-CN. Modification of diet to reduce inflammation had an additional benefit in improving RTL.

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Statement of Authorship

Conceptualization, H.F.L.M., M.A.v.B. and E.C.M, Methodology, H.F.L.M., M.A.v.B. and E.C.M, Investigation, H.F.L.M., S.O., S.G.M., Resources, H.F.L.M., S.O and S.G.M, Data Curation, H.F.L.M., S.O., S.G.M., M.A.v.B. and E.C.M.; Writing – Original Draft Preparation, H.F.L.M., S.O., M.A.v.B. and E.C.M.; Writing – Review & Editing, H.F.L.M., M.A.v.B., S.O., S.G.M., and E.C.M.; Supervision, S.G.M., M.A.v.B. and E.C.M.; Project Administration, H.F.L.M.; Funding Acquisition, H.F.L.M. Harry Freitag Luglio Muhammad (H.F.L.M.), Edwin C. Mariman (E.C.M.), Marleen A. van Baak (M.A.v.B.), Safarina G. Malik (S.G.M) and Sukma Oktavianthi (S.O.).

Conflict of Interest

There is no financial and personal relationship with other people or organizations that could inappropriately influence this work.

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CHAPTER 5

The Effect of a Low Calorie Low Inflammatory Diet on Relative Telomere Length and Mitochondrial DNA Copy Number among Obese Indonesian Adults

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CHAPTER 6

Interactions between SNPs at Pro-inflammatory Genes, Adiposity and C-reactive Protein and Their Association with Colorectal Cancer Risk

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ABSTRACT

Obesity is associated with increased colorectal cancer (CRC) risk that is driven by increased inflammation. This study aimed to evaluate the interaction between variants in inflammation-related genes (*CRP*, *TNFA*, and *IL6*), adiposity, circulating concentration of high sensitive C-reactive protein (hs-CRP; a marker of systemic inflammation) and CRC risk. Participants undergoing endoscopy for bowel symptoms were recruited, adiposity and circulating hs-CRP concentration were measured. Four SNPs were analyzed including those in *CRP* (rs1800947, rs1205), *TNFA* (rs1800629) and *IL6* (rs1800795). Individuals with a prior history of adenomatous polyps (therefore at higher risk of CRC), had higher hs-CRP concentrations ($p=0.012$). Adiposity measures did not differ significantly between normal and higher-risk participants. Variation at *TNFA* (rs1800629) was associated with body mass index ($p=0.007$) and the presence of colorectal polyps ($p=0.002$). We observed that variants in inflammatory genes may influence the relationships between adiposity and systemic inflammation but these influences appeared to be independent of the effect allele. Variation in *TNFA* was associated with adiposity and presence of colorectal polyps. In addition, SNPs at *CRP*, *TNFA* and *IL6* influence the association of adiposity with systemic inflammation.

Keywords: obesity, inflammation, gene variation, CRP, colorectal polyp.

INTRODUCTION

Obesity is associated with increased risk of several non-communicable diseases including cancers [1,2]. Several adiposity measures, including body mass index (BMI), visceral adiposity, and a body shape index (ABSI) predict colorectal cancer (CRC) risk [2-4]. Disturbances in hormones, insulin resistance, growth factors and systemic inflammation are plausible mechanisms through which obesity amplifies CRC risk [5,6]. In particular, excess adipose tissue is associated with increased leptin production, immune cell infiltration and higher production of pro-inflammatory cytokines [7]. This combination leads to a greater inflammatory state in adipose tissue as well as higher concentrations of circulating inflammatory markers in individuals with obesity [8,9].

Low-grade systemic inflammation is induced by several factors including environmental exposures (diet, physical activity, pollutants) and genetics [10], and the obesity-inflammation relationship is influenced by variants in inflammation-related genes. For example, Eiriksdottir et al. [11] reported that variants of the C reactive protein (*CRP*) gene modulate the relationship between BMI and hs-CRP (a marker of systemic inflammation). Similar findings were reported in other studies [12,13]. Systemic inflammation is associated with increased risk of CRC [14] and single nucleotide polymorphisms (SNPs) at inflammatory genes including *CRP* (rs1800947, rs1205), *TNFA* (rs1800629), and *IL6* (rs1800795) are associated with greater CRC risk [15-17].

However, the effects of variants in inflammation-related genes on the relationships between adiposity, inflammation and the development of CRC are less understood. Therefore, we focused on specific SNPs in *CRP* (rs1800947, rs1205), *TNFA* (rs1800629) and *IL6* (rs1800795) known to affect circulating concentrations of the respective proteins [18-20], and for which meta-analyses have shown associations with increased risk of CRC and other cancers [15-17]. We aimed to investigate associations between each of these SNPs and i) measures of adiposity and ii) plasma concentrations of hs-CRP as a marker of systemic inflammation. We also examined whether those SNPs were associated with the risk of colorectal adenomatous polyps (a CRC precursor). Lastly, we evaluated the influence of the selected SNPs on the correlation between adiposity and hs-CRP.

METHODS

Study design

We used a cross-sectional study design involving adults living in the North East of England, United Kingdom, who participated in the Biomarkers Of Risk In Colorectal Cancer (BORICC) Study [21]. Adult (>18 years) participants who were listed to undergo endoscopy for diagnostic purposes were recruited from Wansbeck General Hospital, Northumberland, UK. The exclusion criteria of this study included: a familial predisposition to CRC; colorectal neoplasia and inflammatory disease. Ethical

approval for the study was received from the Northumberland Local Research Ethics Committee (Project reference NLREC2/2001) and details of participant recruitment have been described elsewhere [21]. A total of 329 participants were recruited, comprising healthy individuals (n=236, at normal risk of CRC) and those with colorectal adenomatous polyps (n=93, at higher risk of CRC).

Markers of adiposity and inflammation

Anthropometrics was assessed as body weight (kg), body mass index (BMI; kg/m²), waist circumference (WC; cm), hip circumference (cm), and waist-to-hip ratio (WHR) by trained staff using standardized protocols. ABSI was calculated from WC, BMI and height measurements according to Krakauer and Krakauer (ABSI: WC/((BMI^(2/3)×height^(1/2)))[22]. Information on participant characteristics including medical history, drug history, smoking status, and supplement use were collected using a questionnaire. Plasma concentrations of hsCRP were measured using an enzyme linked immunosorbent assay.

Assessment of genetic variants

From plasma that had been stored at -80°C, DNA was extracted using the ThermoFisher Genomic DNA isolation kit (Waltham, MA, USA) following the manufacturer's instructions. The DNA was sent to LGC, Biosearch Technologies for analysis of genetic variants using KASP assays (LGC group) for the following 4 SNPs in inflammation-related genes: *CRP* (+942G>C, rs1800947 and +1846C>T, rs1205), *TNFA* (-308 G>A, rs1800629) and *IL6* (-174G>C, rs1800795).

The variation at *TNFA* rs1800629, *IL6* (rs1800795) and *CRP* (rs1205) lies outside of the coding region whilst variation at *CRP* (rs1800947) is within the coding region. The G allele of *TNFA* (rs1800947), C allele of *IL6* (rs1800795), G allele of *CRP* (rs1800947) and C allele of *CRP* (rs1800947), were considered as effect alleles because they have been associated with increased expression or concentration of the encoded proteins [23-27]. For further analyses, we used a dominant model (AA vs Aa+aa) comparing those who were homozygous for the effect allele with heterozygotes and those homozygous for the non-effect allele. To evaluate the cumulative effect of gene variants on risk of adenomatous polyps, we calculated a genetic risk score (GRS) for those with complete data for all four SNPs. Using the available literature (Table 6.2) [23-27], a score of 0 was allocated to those carrying 0 or 1 effect allele, and a score of 1 was allocated to those carrying two effect alleles (homozygote). The score for each gene was summed to yield a 'total score' ranging between 0 and 4.

Data analysis

Statistical analyses were performed using JASP software Version 0.8.3.1 (The University of Amsterdam). Differences in adiposity and hs-CRP concentration between those with and without adenomatous polyps were analyzed using the Mann-Whitney test. Calculation of Hardy-Weinberg equilibrium was performed using the online tool snptest.com [28]. Differences in adiposity measures and in hs-CRP concentrations between genotypes were analyzed using the Mann-Whitney test and ANCOVA with adjustment for age, sex, medical history (including diabetes mellitus, any types of cancer, heart diseases, irritable bowel syndrome, fatty liver disease), drug history, smoking status, and presence of adenomatous polyps. Those factors were previously reported to be associated with inflammation [10]. The odds ratio (OR) for risk of adenomatous polyps, according to SNPs at inflammatory genes, was calculated using conditional logistic regression. Interactions between SNPs at inflammatory genes, adiposity measures, and hs-CRP concentrations for all participants combined were analyzed using linear regression. Within each specific genotype group, relationships between adiposity measures and hs-CRP concentration were investigated using linear regression with adjustment for age, sex, medical history, drug history, smoking status, and polyps group.

RESULTS

Participant characteristics are summarized in Table 6.1. From a total of 408 potential participants, we used data from 329 who had a complete dataset including genotype. Overall, there were similar numbers of males and females (169 and 160, respectively) who ranged in age from 17-83 years. Individuals with adenomatous polyps were more likely to be male and older compared with healthy individuals (without polyps). There were no significant differences in adiposity measures between those with, and those without, polyps. Participants with polyps had a higher WHR, but this association was no longer significant after adjustment for potential confounding factors. Systemic inflammation, assessed as circulating concentration of hs-CRP, was higher in participants with polyps ($p=0.012$). Participants with adenomatous polyps had higher GRS for inflammatory genes than those without adenomatous polyps ($p=0.002$).

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Table 6.1. Characteristics of study participants.

	All participants	With adenomatous polyp (n=93)		Without adenomatous polyp (n=236)			p*	p^	
		Men	Women	p ^o	Men	Women			p ^o
		(n=63)	(n=30)		(n=106)	(n=130)			
Age (years)	53.1±13.9	60.6±11.7	60.1±11.4		48.1±13.1	51.9±13.9	<0.001		
Height (m)	1.69±0.10	1.74±0.09	1.59±0.06	<0.001	1.77±0.08	16.2±0.07	<0.001	0.113	
Weight (kg)	82.2±19.1	90.1±19.5	72.9±17.2	<0.001	87.9±17.7	75.7±17.4	<0.001	0.129	
BMI (kg/m ²)	28.6±5.8	29.6±6.1	28.7±6.2	0.424	27.9±4.6	28.7±6.3	0.890	0.182	
WC (cm)	94.5±15.6	102.1±14.0	87.0±21.4	<0.001	98.3±13.2	89.5±14.2	<0.001	0.050	
HC (cm)	104.4±13.0	104.6±11.1	99.8±22.6	0.121	102.9±9.4	106.6±13.3	0.251	0.228	
WHR	0.90±0.09	0.98±0.06	0.87±0.07	<0.001	0.95±0.07	0.84±0.07	<0.001	<0.001	
ABSI	0.078±0.007	0.081±0.007	0.076±0.006	<0.001	0.080±0.005	0.075±0.006	<0.001	0.010	
hs-CRP (mg/L)	3.90±5.06	5.08±6.40	5.31±5.4	0.814	2.78±3.4	3.62±4.98	0.571	0.003	
GRS	2.3±0.87	2.6±0.8	2.8±0.6	0.973	2.14±0.84	2.19±9.2	0.271	<0.001	

Data are presented as mean ± standard deviation; ^oChi-square test; *ANOVA test between those with and without polyp (combined men and women); ^ANCOVA test between those with and without polyp (combined men and women) adjusting for age, sex, current smoking status, supplement use, medical diagnosis, and inflammation affecting-drug use, ^oANCOVA test between men and women adjusting for age, current smoking status, supplement use, medical diagnosis, and inflammation affecting-drug use; ABSI: A body shape index; BMI: body mass index, GRS: genetic risk score, hs-CRP : high-sensitivity C-reactive protein, HC: hip circumference, WC: waist circumference, WHR: waist-to-hip ratio.

The distribution of variants in inflammation-related genes is shown in Table 6.2. In this study, not all genotypic data were available for all participants due to call rate. The call rates for genetic analyses were between 76% (rs1205) and 96% (rs1800947).

Table 6.2. Characteristics of genetic variants in study participants.

Gene	SNP	Allele	Amino acid codon	SNP location	Effect	% call rate	p HWE	MAF
<i>TNFA</i>	rs1800629	G>A	No	Upstream Transcript Variant	G allele increase TNF-alpha activity and plasma concentration [23]	78	0.027	A (0.15)
<i>IL6</i>	rs1800795	C>G	No	Upstream Transcript Variant	C allele increases IL-6 transcription IL-6 concentration in blood [24-26]	85	0.001	C (0.41)
CRP	rs1800947	G>C	L [CTG] > L [CTC]	Coding Sequence Variant	G allele increases CRP concentration [27]	96	0.047	C (0.03)
CRP	rs1205	C>T	No	Untranslated Region variant	C allele increase CRP concentration [27]	76	0.026	T (0.34)

HWE: Hardy-Weinberg equilibrium; MAF : minor allele frequency; CRP : C-reactive protein; TNFA : tumor necrosis factor alpha; IL6 : interleukin 6; % call rate : % successfully analyzed genotypes from all available DNA samples.

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Differences in markers of adiposity and in hs-CRP concentration between genotypes of each inflammation-related gene are summarized in Table 6.3. Those carrying the A allele of *TNFA* (rs1800629) had a lower body weight ($p=0.023$) and lower BMI ($p=0.007$) compared with those carrying the G allele. In contrast, for *IL6* (rs1800795), those carrying the G allele had higher body weight ($p=0.047$). Circulating hs-CRP concentrations were lower in those with the A allele of *TNFA* (rs1800629) ($p=0.023$) or the T allele of *CRP* (rs1205) ($p=0.007$); however these findings were no longer statistically significant after adjusting for polyps status. There were no differences in markers of adiposity between *CRP* (rs1205) genotypes or between *CRP* (rs1800947) genotypes, and the latter did not influence hs-CRP concentration (all $p>0.05$).

Table 6.3. Differences in markers of adiposity and of hs-CRP concentration between genotypes for inflammation-related genes.

<i>TNFA</i> (rs1800629)					
	G/G (n=188)	G/A + A/A (n=68)	Model 1 p*	Model 2 p ^a	Model 3 p ^b
Weight (kg)	84.1±20.2	79.0 ± 16.7	0.061	0.015	0.023
BMI (kg/m ²)	29.4±6.2	26.9 ± 4.8	0.004	0.003	0.007
Waist circumference (cm)	95.6±17.0	92.5 ± 14.7	0.187	0.079	0.086
Hip circumference (cm)	105.6±14.6	103.3 ± 10.5	0.231	0.160	0.115
WHR	0.90±0.09	0.89 ± 0.09	0.387	0.129	0.224
Hs-CRP (mg/L)	4.4±5.4	2.5 ± 3.9	0.023	0.092	0.402
ABSI	0.078±0.007	0.078±0.006	0.783	0.845	0.837
<i>IL6</i> (rs1800795)					
	C/C (n=60)	G/C + G/G (n=219)	p*	p ^a	p ^b
Weight (kg)	79.8 ± 16.4	82.7 ± 19.6	0.300	0.045	0.047
BMI (kg/m ²)	27.9 ± 4.7	28.8 ± 6.0	0.271	0.073	0.077
Waist circumference (cm)	93.2 ± 12.6	94.5 ± 16.4	0.320	0.164	0.165
Hip circumference (cm)	103.0 ± 8.9	104.9 ± 13.8	0.307	0.100	0.099
WHR	0.90 ± 0.09	0.89 ± 0.09	0.741	0.928	0.928
Hs-CRP (mg/L)	4.1 ± 4.8	3.8 ± 5.2	0.727	0.911	0.846
ABSI	0.078±0.007	0.078±0.007	0.817	0.278	0.275
<i>CRP</i> (rs1800947)					
	G/G (n=294)	G/C+C/C (n=20)	p*	p ^a	p ^b
Weight (kg)	82.4 ± 19.6	81.3 ± 15.9	0.813	0.502	0.504
BMI (kg/m ²)	28.6 ± 5.9	27.9 ± 5.4	0.603	0.467	0.468
Waist circumference (cm)	94.6 ± 16.0	92.5 ± 12.8	0.575	0.589	0.590
Hip circumference (cm)	104.4 ± 13.4	103.8 ± 12.0	0.846	0.646	0.643
WHR	0.91 ± 0.09	0.89 ± 0.08	0.513	0.774	0.778
Hs-CRP (mg/L)	4.0 ± 5.2	2.4 ± 2.0	0.237	0.392	0.353
ABSI	0.078±0.007	0.076±0.005	0.170	0.380	0.379
<i>CRP</i> (rs1205)					
	C/C (n=116)	C/T+T/T (n=135)	p*	p ^a	p ^b
Weight (kg)	82.5 ± 19.6	82.0 ± 17.2	0.821	0.963	0.904
BMI (kg/m ²)	28.5 ± 6.0	28.5 ± 5.0	0.985	0.910	0.791
Waist circumference (cm)	95.1 ± 14.8	94.2 ± 14.2	0.625	0.787	0.875
Hip circumference (cm)	104.7 ± 11.4	104.9 ± 10.8	0.849	0.978	0.998
WHR	0.91 ± 0.09	0.89 ± 0.09	0.376	0.637	0.843
Hs-CRP (mg/L)	4.9 ± 6.5	2.9 ± 3.6	0.007	0.037	0.074
ABSI	0.078±0.007	0.078±0.007	0.390	0.450	0.484

Data are presented as mean ± standard deviation, *Model 1 basic ANOVA, ^aModel 2: As for Model 1 adjusted for age, sex, medical history, drug history, supplement use, smoking status; ^bModel 3: As for Model 2 plus adjustment for presence of adenomatous polyps; BMI: body mass index, hs-CRP : high-sensitivity C-reactive protein, WHR: waist-to-hip ratio, ABSI: a body shape index

Conditional logistic regression was used to evaluate the role of SNPs in inflammation-related genes on the risk of adenomatous polyps. We observed increased OR for the development of polyps among those who carried the GG genotype of *TNFA* (rs1800629) and CC genotype of *CRP* (rs1205). When corrected for confounding factors including age, sex, diagnoses of other diseases, anti-inflammatory drug use, supplement use, smoking status, BMI and C-reactive protein, the effect of the GG genotype of *TNFA* (rs1800629) on risk of adenomatous polyps remained statistically significant ($p=0.002$), while the effect of CC genotype of *CRP* (rs1205) diminished ($p=0.255$) (Table 6.4).

Table 6.4. Odds Ratio (OR) for risk of adenomatous polyp according to genotype of inflammation-related genes.

	N	Risk genotype ^a	Analysis	OR*	95% CI*	p*	OR [†]	95% CI [†]	p [†]
<i>TNFA</i> (rs1800629)	258	GG	GG vs GA+AA	4.46	1.82 - 10.90	0.001	9.33	2.33 - 37.38	0.002
<i>IL6</i> (rs1800795)	282	CC	CC vs GC+GG	1.05	0.56 - 1.98	0.885	0.77	0.34 - 1.76	0.540
<i>CRP</i> (rs1800947)	317	GG	GG vs GC+CC	1.62	0.53 - 4.98	0.401	0.75	0.14 - 2.60	0.499
<i>CRP</i> (rs1205)	254	CC	CC vs CT+TT	2.00	1.13 - 3.54	0.017	1.53	0.74 - 3.17	0.255

^aDominant model of risk allele, see Table 6.2. *Logistic regression analysis without adjustment; [†]Conditional logistic regression with adjustment for age, sex, medical diagnosis, anti-inflammatory drug use, supplement use, smoking status, body mass index and C-reactive protein.; OR=adjusted odds ratio

The stratified analysis on the relationships between measures of adiposity and hs-CRP concentration based on variation in inflammation-related genes is summarized in Table 6.5. Among those with the GG genotype of *TNFA* (rs1800629), adiposity measures (body weight, BMI, waist and hip circumference) correlated positively with hs-CRP concentration ($p < 0.05$). This correlation was not seen among those with GA+AA genotypes (all $p > 0.05$). For IL-6, there were no relationships between adiposity markers and hs-CRP concentration among those with the C/C genotype (rs1800795). In contrast, for those with the C/G + G/G genotype (rs1800795), body weight, BMI, waist and hip circumference were associated with hs-CRP concentration (all $p < 0.05$). Among those with G/G genotype of *CRP* (rs1800947), hs-CRP was correlated with all adiposity measures while no interaction was seen among those with G/C+C/C genotypes. With respect to the *CRP* gene (rs1205), adiposity measures were associated with hs-CRP concentrations only in participants with the T/C + T/T genotypes with the C/C genotype. Interestingly, for all genotypes individually, there was no relationship between ABSI and hs-CRP concentration (Table 6.5).

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Table 6.5. Influence of genotype for inflammation-related genes on the relationships between markers of adiposity and hs-CRP concentration.

	Correlation of adiposity markers with plasma concentration of hs-CRP							
	G/G				G/A + A/A			
	Model 1		Model 2		Model 1		Model 2	
TNFA (rs1800629)	B	p	B	p	B	p	B	p
Body weight (kg)	0.303	0.002	0.294	0.002	0.177	0.381	0.125	0.553
BMI (kg/m ²)	0.258	0.003	0.244	0.004	0.176	0.315	0.150	0.397
Waist circumference (cm)	0.211	0.031	0.215	0.025	0.318	0.145	0.269	0.246
Hip circumference (cm)	0.195	0.022	0.209	0.012	0.096	0.585	0.053	0.773
WHR	0.057	0.664	0.037	0.776	0.462	0.061	0.421	0.100
ABSI	-0.016	0.887	-0.001	0.995	0.163	0.485	0.104	0.668
	C/C				C/G + G/G			
IL6 (rs1800795)	B	p	B	p	B	p	B	p
Body weight (kg)	0.136	0.475	0.136	0.480	0.368	<0.001	0.350	<0.001
BMI (kg/m ²)	0.172	0.316	0.165	0.346	0.367	<0.001	0.344	<0.001
Waist circumference (cm)	0.280	0.189	0.287	0.178	0.313	<0.001	0.307	<0.001
Hip circumference (cm)	0.221	0.197	0.223	0.193	0.266	<0.001	0.288	<0.001
WHR	0.178	0.500	0.187	0.480	0.189	0.098	0.124	0.277
ABSI	0.201	0.302	0.239	0.241	0.012	0.908	0.003	0.972
	G/G				G/C + C/C			
CRP (rs1800947)	B	p	B	p	B	p	B	p
Body weight (kg)	0.327	<0.001	0.316	<0.001	0.035	0.946	0.052	0.940
BMI (kg/m ²)	0.329	<0.001	0.316	<0.001	0.024	0.967	0.017	0.982
Waist circumference (cm)	0.292	<0.001	0.284	<0.001	-0.015	0.987	0.029	0.984
Hip circumference (cm)	0.246	<0.001	0.251	<0.001	-0.227	0.546	-0.460	0.509
WHR	0.193	0.050	0.164	0.097	0.696	0.322	1.307	0.253
ABSI	-0.016	0.835	0.003	0.966	-0.260	0.599	-0.259	0.683
	C/C				T/C+T/T			
CRP (rs1205)	B	p	B	p	B	p	B	p
Body weight (kg)	0.277	0.030	0.235	0.057	0.415	<0.001	0.415	<0.001
BMI (kg/m ²)	0.245	0.032	0.197	0.079	0.460	<0.001	0.460	<0.001
Waist circumference (cm)	0.264	0.041	0.206	0.099	0.485	<0.001	0.486	<0.001
Hip circumference (cm)	0.288	0.009	0.254	0.017	0.411	<0.001	0.414	<0.001
WHR	0.086	0.597	0.015	0.924	0.324	0.017	0.330	0.018
ABSI	-0.015	0.913	-0.008	0.950	0.174	0.164	0.173	0.171

Model 1: Linear regression adjusting for age, sex, medical diagnosis, drug, supplement use and smoking status; Model 2: as for Model 1 with additional adjustment for presence of adenomatous polyps, BMI: body mass index, WHR: waist-to-hip ratio, ABSI: a body shape index

DISCUSSION

This study investigated the interaction between variants of inflammatory genes (*CRP*, *TNFA*, and *IL6*), markers of adiposity, systemic inflammation (assessed using hs-CRP concentration) and CRC risk as indicated by the presence of adenomatous polyps. People with colorectal polyps had higher hs-CRP concentrations but did not differ in adiposity measures from the healthy controls. Findings from previous studies have been inconsistent; for example, in the Calcium Polyp Prevention Study, there was no

association between hs-CRP concentration and occurrence of colorectal adenomas [29]. In contrast, several studies have reported that hs-CRP is positively associated with the presence of colorectal adenomas [30,31].

We found no differences in body weight or BMI between those with, and without, colorectal polyps. These findings differ from the conclusion that obesity is associated with colorectal adenoma risk reported in an earlier meta-analysis [32]. In contrast, in the present study, markers of abdominal adiposity, including WHR and ABSI, were associated with the presence of colorectal polyps. This finding supports the report from the Black Women's Health Study which showed that increased ABSI was associated with increased adenoma risk [33].

All four SNPs in three inflammatory genes have been associated with greater CRC risk in other studies [15-17]. In the present study, we showed that the GRS from a combination of SNPs at inflammatory genes was significantly higher among those with adenomatous polyps. However, when the analysis was done for each SNP individually, only participants with the GG genotype for *TNFA* (rs1800629) were more likely to have an adenomatous polyps. In addition, only the *TNFA* (rs1800629) genotype was associated with adiposity.

We observed that variants in inflammatory genes may influence the relationship between adiposity and systemic inflammation. However, there was no consistent relationship between carriage of the effect allele, i.e. the allele associated with increased plasma concentrations of the corresponding inflammatory proteins, and the likelihood that adiposity measures would correlate with hs-CRP concentration. For those with the G/G genotype of *TNFA* (rs1800629), C/G + G/G genotype of *IL6* (rs1800795), G/G genotype of *CRP* (rs1800947) and T/C+T/T genotype of *CRP* (rs1205), there were significant relationships between adiposity measures and hs-CRP concentrations. This relationship was not seen in the opposite genetic group, suggesting that genetic variation influences the pro-inflammatory effects of increased adiposity. Across all genotypes individually, there was no relationship between ABSI and hs-CRP concentration. This is in contrast with a study of 1442 obese and overweight adults in Central China where there was a weak positive correlation between ABSI and hs-CRP concentration [34].

The findings of this study are summarized conceptually in Figure 6.1. This is in the context of evidence that lifestyle factors that increase adiposity, together with genetic factors, amplify the risk of developing CRC [35]. In the present study, we showed that BMI was not associated with risk of colorectal polyps, which are precursors of CRC, whereas WHR and ABSI (indicators of central obesity) were higher among those with colorectal polyps. The influence of central obesity on colorectal cancer risk has been reported in a recent meta-analysis of prospective studies [36].

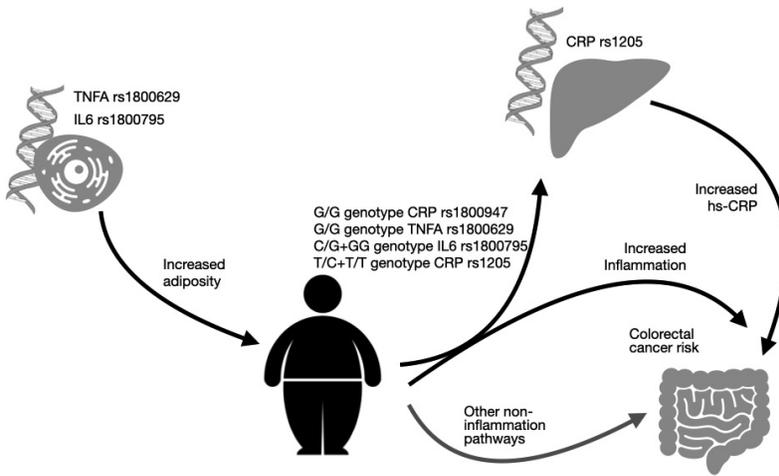


Figure 6.1. Proposed relationships between genotypes for variants in pro-inflammatory genes, adiposity, inflammation and colorectal cancer risk. hs-CRP: high sensitive C reactive protein. Among the four genetic variants investigated in this study, only SNPs at *TNFA* and *IL6* were associated with adiposity measures. For those with the G/G genotype of *TNFA* (rs1800629), C/G + G/G genotype of *IL6* (rs1800795), G/G genotype of *CRP* (rs1800947) and T/C+T/T genotype of *CRP* (rs1205), there were significant relationships between adiposity measures and hs-CRP concentrations. The increment of hs-CRP thus increase risk of colorectal polyps.

Among the four genetic variants investigated in this study, only SNPs at *TNFA* and *IL6* were associated with adiposity measures. Individuals with the G/G genotype of *TNFA* had higher body weight and BMI and these associations remained significant after extensive adjustment for potential confounders. This is consistent with previous reports that the GG variant of the *TNFA* gene is associated with greater obesity risk [37]. The G allele of *TNFA* (rs1800629) is associated with increased TNF-alpha protein in the circulation [23] and obesity and TNF-alpha concentration are correlated positively [38,39]. However, the mechanism through which the GG variant of the *TNFA* gene may increase obesity risk remains to be discovered.

Variants in *TNFA* (rs1800629) and *CRP* (rs1205) were associated with hs-CRP concentration in unadjusted analyses but these associations were weakened after adjustment for potential confounding factors. Results from this study are in line with results from other studies [23,27,40]. A recent meta-analysis reported no significant correlation between *TNFA* (rs1800629) genotype and CRC risk [41]. In contrast, in the present study, we observed that those carrying the G/G genotype of *TNFA* (rs1800629) had increased risk of adenomatous polyps. TNF-alpha is an inflammatory

mediator that drives cancer progression [42]. Those with the G/G genotype of *TNFA* (rs1800629) have higher plasma concentration of TNF-alpha [24] that may increase risk of colorectal polyps development.

Potential limitations of this study include that the study participants were selected from those invited for a colorectal examination for gastrointestinal symptoms which may limit the generalisability of findings to a wider population, this is reflected by the Hardy-Weinberg equilibrium value. Secondly, we used a simple GRS for pro-inflammatory genes with the assumption that all gene variants had equal effects. Since each individual gene variation is likely to have a different effect size, use of a weighted approach for derivation of the GRS may have been advantageous [43]. However, with the relatively small number of genetic variants included in our score, we did not consider this more sophisticated approach was justified. A strength of this study is that we evaluated data from those with colorectal adenomatous polyps compared with those who underwent a colorectal examination and who were shown to be polyps-free (healthy participants). This minimized the likelihood of misclassification of participants. Finally, in addition to widely-used markers of adiposity, we investigated relationships with ABSI, a novel measure of central adiposity that is associated with CRC risk [4].

In summary, this study showed that genetic variation in inflammatory markers influenced individual response to adiposity. Variation in *TNFA* was associated with adiposity and the presence of colorectal polyps. In addition, SNPs at *CRP*, *TNFA* and *IL6* influence the association of adiposity with systemic inflammation. These findings require confirmation in additional population-based studies. Finally, from a cancer prevention perspective, it will be important to discover whether the effects of weight loss on inflammatory markers is influenced by these genetic variants.

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THE INTERACTION

BETWEEN INFLAMMATORY
PROPERTIES OF DIET AND
GENETIC VARIATION IN

BODY WEIGHT REGULATION

CHAPTER 7

The Interaction of Inflammatory Gene Variations with the Association between Diet, hs-CRP, and Weight Regain in the DiOGenes Trial

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ABSTRACT

The objective of this study was to evaluate the interaction between gene variations in pro-inflammatory genes (*CRP*, *TNFA*, and *IL6*) and diet and its effect on hs-CRP and weight regain following weight loss in the DiOGenes Trial. 453 participants with overweight or obesity of the DiOGenes trial, who successfully finished the weight loss and weight maintenance phases and in whom gene variations were measured, were included. During the weight maintenance phase, subjects consumed an *ad libitum* diet with different recommendations on protein content and glycemic index (GI). Variations in the pro-inflammatory genes *CRP* (rs1205 and rs1800947), *TNFA* (rs1800629 and rs361525) and *IL6* (rs1800795) were evaluated. Variations in the *CRP* gene were associated with hs-CRP concentration at baseline and during weight maintenance. Variations in pro-inflammatory genes were not associated with body weight at baseline, after weight loss and at the end of the weight maintenance period (all $p > 0.05$). The positive correlation between GI and changes in body weight during weight maintenance was significant among those with the GG genotype ($p = 0.044$) of *TNFA* rs1800629 but not in those with the GA+AA genotype ($p = 0.487$). Percent protein intake was negatively correlated with changes of body weight only among those with the CC genotype of *CRP* rs1205 ($p = 0.016$), CC genotype of *CRP* rs1800947 ($p = 0.028$) and the GC+GG genotype of *IL6* rs1800795 ($p = 0.004$). Variations in pro-inflammatory genes (*CRP*, *TNFA*, and *IL6*) influenced the weight regain response to the protein content and GI of the diet.

Keywords: Obesity, weight loss, genetic variation, inflammation, diet.

INTRODUCTION

Obesity is associated with increased systemic and adipose tissue inflammation due to immune cell infiltration and higher production of pro-inflammatory cytokines [1]. An increase in high-sensitive C reactive protein (hs-CRP) in obesity has been shown in population-based studies [2]. Among individuals with obesity and overweight, weight loss is associated with a reduction in hs-CRP [3-6]. Changes in hs-CRP were positively correlated with changes in body weight and body fat over a 2-year period in the POUNDS LOST study [3]. In contrast to systemic inflammation, studies showed a significant increase of inflammation markers in adipose tissue immediately after a period of weight loss [7-9], and these increments were associated with subsequent weight regain. It has been argued that adipose tissue inflammation may play a role in the regulation of body weight after weight loss [10].

DiOGenes is an intervention study that consisted of a weight loss intervention and dietary modification (focusing on protein content and glycemic index (GI) of the diet) after weight loss for weight loss maintenance, and was conducted in 8 countries in Europe with an objective to evaluate factors associated with weight regain [11]. It was shown that diets with a low glycemic index (GI) were associated with less weight regain and a reduction of inflammation. Higher protein diets were also associated with less weight regain, but with less reduction of inflammation [4,12]. These findings suggest an interaction between diet, inflammation, and weight regain, which is not fully understood. Because inflammation is influenced by gene variation [13-16], analysis of variations in inflammatory genes might partly explain the role of inflammation in weight regain.

This study therefore aimed to evaluate the role of variations in pro-inflammatory genes on the associations between protein intake, glycemic index (GI), inflammation, and weight regain after a weight loss program in adults with overweight or obesity. Variations in genes encoding *CRP*, *TNFA*, and *IL6* were selected because those were previously reported to have an effect on circulating hs-CRP as well as obesity in humans [13-16].

METHODS

Study Participants

The DiOGenes study is a randomized controlled trial conducted among families in which at least one of the parents was overweight/obese. Centers in 8 countries across Europe participated in this study: The Netherlands, Denmark, United Kingdom, Greece, Spain, Germany, Bulgaria, and the Czech Republic. The inclusion and exclusion criteria for the families have been described in detail before [17]. The 453 adults which were included in this secondary analysis were less than 65 years old and were overweight or obese (body mass index between 27 kg/m² and 45 kg/m²) at baseline. They had all completed the weight loss and 6-month weight maintenance phase and genotyping of their DNA was available.

The Study Design

This study was done in 2 phases: the weight loss phase and the weight maintenance phase. During weight loss phase subjects followed an 8-week low-calorie diet of 800 kcal/day. The diet in this phase was based on Modifast meal replacements (Nutrition & Santé) and roughly 200 grams of vegetables per day. During the weight loss period, subjects were routinely checked for compliance and adverse effects. After 8 weeks, those who achieved 8% of weight loss from their initial weight were eligible for the weight maintenance phase.

During the 26-week weight maintenance phase, subjects were randomized to 5 different diets with variations of protein composition and glycemic index (GI): 1) high protein, low GI; 2) high protein, high GI; 3) low protein, low GI; 4) low protein, high GI and 5) control. Details of the diet composition in the different groups and the way the intervention was conducted have been reported elsewhere [18]. In short, recommended protein content in the low protein groups was 13 % of energy intake and 25% in the high protein groups. A difference of 15 glycemic index units was targeted between the low and high GI groups. In the control group, subjects received recommendations according to the standard healthy diet recommendation in their own country. The recommended fat intake in all groups was 25-30% of total energy intake. There was no restriction on the total energy intake (*ad libitum*). For the purpose of the analysis in this paper, we merged subjects from all dietary groups and evaluated the trial as an observational study.

Dietary Assessment

Subjects reported their dietary intake using a dietary record of 3 consecutive days. This dietary assessment was done three times : at screening (baseline), at weeks 4 and at week 26 during the weight maintenance phase. Data on food intake was then translated into the intake of nutrients based on the local food composition databases. Analysis of the glycemic index was done as previously described [19]. Protein and glycemic index during the weight maintenance phase were the average of intakes at week 4 and 26 of the weight maintenance phase or of week 26 alone when week 4 data were missing.

Anthropometric and Inflammation assessment

Anthropometric measurements such as body weight and body composition were done at baseline, at the end of the weight loss phase and at the end of the weight maintenance phase. Body composition was measured either by dual-energy X-ray absorptiometry (DXA) or bioelectrical impedance analysis (BIA). Hs-CRP was measured as a marker of systemic inflammation. Serum hs-CRP was analysed using an immunoturbidimetric assay (Roche Diagnostics). Details regarding the hs-CRP analysis have been reported previously [4].

Genotyping

DNA for genotyping was extracted from buffy coats of blood specimens collected in EDTA-tubes. Analysis of genotypes was done using the Illumina Bead Station System (IlluminaInc) by IntegraGen. Five single nucleotide polymorphisms from 3 genes (*CRP* (rs1205 and rs1800947), *TNFA* (rs1800629 and rs361525) and *IL6* (rs1800795)) were selected because of their previous involvement in the regulation of inflammation in humans. Based on the literature, we selected the effect allele as the allele that increased expression or inflammation level (Table 7.1). The analysis was done with the dominant model for the effect allele, except for *CRP* rs1800947 because the number of the homozygote effect genotype (GG) was limited, thus we combined the heterozygotes with the homozygotes of the minor allele (GG+GC). Hardy Weinberg equation analysis was done using SNPstat (<https://www.snpstats.net>).

Table 7.1. The effect of selected gene variations in pro-inflammatory genes.

Gene	Gene variation	Allele Variation	Amino acid codon	Location	Effect allele	Effect
<i>CRP</i>	rs1205	C>T	No	Untranslated Region variant	C	C allele increases hs-CRP level [13]
<i>CRP</i>	rs1800947	G>C	L [CTG] > L [CTC]	Coding Sequence Variant	G	G allele increases hs-CRP level [13]
<i>TNFA</i>	rs1800629	G>A	No	Upstream Transcript Variant	G	G allele increases TNF-alpha activity and plasma level [21]
<i>TNFA</i>	rs361525	G>A	No	Promoter region	G	G allele increases TNF-alpha expression [22]
<i>IL6</i>	rs1800795	C>G	No	Upstream Transcript Variant	C	C allele increases <i>IL6</i> transcription and IL-6 level in blood [23-25]

Statistical Analysis

The statistical analysis was done using JASP software Version 0.8.3.1 (University of Amsterdam) [20]. The genetic dominant model was used when comparing the effect of genotypes on anthropometric measures, systemic inflammation (plasma hs-CRP). The effect of *CRP* (rs1205 and rs1800947), *TNFA* (rs1800629 and rs361525) and *IL6* (rs1800795) gene variations on body weight, body composition and hs-CRP was analysed at baseline using independent t-tests. ANCOVA was used to evaluate the association between gene variations and changes in body weight and hs-CRP during weight loss (all groups) with correction for age and gender. A linear regression test was used to analyze the influence of gene variations (*CRP*, *TNFA*, and *IL6*) on the correlation between changes in hs-CRP and body weight during weight maintenance with correction for age, gender and study center. Factors associated with weight regain and hs-CRP were used as confounding factors. Age was used as confounding factor because it was associated with hs-CRP in the literature [21] and in this study

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gender and study center were associated with weight regain. A linear regression test was also used to evaluate the correlation between dietary GI and percent protein during weight maintenance and weight changes based on gene variations with correction for gender, study center, BMI at randomization. For GI analysis, additional correction for protein was done. For protein analysis an additional correction for GI was done.

RESULTS

Details about allele selection and genotype grouping are presented in Table 7.1. Allele frequencies and Hardy Weinberg equilibrium are shown in Supplementary Table 7.1. Subjects were grouped based on their genotypes which include included *CRP* (rs1205 and rs1800947), *TNFA* (rs1800629 and rs361525) and *IL6* (rs1800795). We analyzed data of all subjects with a dominant model, except for *CRP* rs1800947, because analysis with other models was hindered by the number of subjects in each genetic group.

In Table 7.2 we show that variations in the *CRP* gene (rs1205 and rs1800947) were associated with differences in baseline hs-CRP concentration ($p=0.010$ and $p=0.030$, respectively). However, these gene variations were not associated with differences in anthropometric measures at baseline. Differences in changes in body weight and hs-CRP between genotype groups were not seen during the weight-loss period (Table 7.3). Changes in body weight also did not differ between genotypes during the weight maintenance phase. However, the GC + GG genotypes of *CRP* (rs1800947) were associated with a greater reduction in hs-CRP than the CC genotype during the weight maintenance period (Table 7.3).

Table 7.2. Baseline anthropometric variables and hs-CRP in genotypes of the *CRP*, *TNFA* and *IL6* genes.

Baseline values	CRPRS1205			CRPRS1800947			TNFA RS1800629			TNFA rs361525 gen			IL6 rs1800795		
	C/C n=357	C/T+T/T n=412	p [^]	C/C n=683	G/C+G/G n=86	p [^]	G/G n=564	G/A+A/A n=205	p [^]	G/G n=685	G/A+A/A n=84	p [^]	C/C n=132	G/C+GG n=637	p [^]
Age (years)	41.2±6.3	41.3±6.2	0.83	41.3±6.2	41.0±6.2	0.45	41.5±6.3	40.6±6.2	0.05	41.2±6.3	41.6±5.9	0.62	41.7±6.9	41.2±6.1	0.28
Height (m)	2.0±0.1	2.0±0.1	0.39	2.0±0.1	2.0±0.1	0.38	2.0±0.1	2.0±0.1	0.94	2.0±0.1	2.0±0.0	N/A	2.0±0.0	2.0±0.1	N/A
Weight (kg)	99.3±17.6	100.0±17.6	0.81	99.5±17.8	100.8±15.6	0.44	99.7±17.8	99.6±17.1	0.87	99.7±17.6	99.2±18.0	0.67	100.0±19.3	99.6±17.2	0.94
BMI (kg/m ²)	34.5±5.0	34.5±4.8	0.94	34.5±4.9	34.6±4.8	0.90	34.5±4.9	34.5±5.0	0.99	34.5±4.9	34.5±4.9	0.87	34.5±5.1	34.5±4.8	0.86
Body fat (%)	40.5±8.1	40.5±7.5	0.72	40.7±7.8	39.2±7.8	0.16	40.4±8.0	40.8±7.2	0.38	40.4±7.8	41.0±7.6	0.57	41.0±7.9	40.4±7.8	0.63
hs-CRP	4.6±4.2	4.0±3.7	0.01	4.4±3.9	3.7±3.8	0.03	4.4±4.2	3.9±3.2	0.61	4.3±3.9	4.4±3.8	0.86	4.2±3.8	4.3±4.0	0.83

[^]Mann Withney test; Data are presented as mean ± standard deviation.

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Table 7.3. Changes in body weight and hs-CRP during the low calorie and weight maintenance periods in the selected genotype groups.

	Weight Loss Period						Weight Maintenance Period					
	N	Body weight (kg)	p*	N	hs-CRP (mg/L)	p*	N	Body weight (kg)	p*	N	hs-CRP (mg/L)	p*
CRP (rs1205)												
C/C	305	-11.2±0.2	0.53	288	-1.0±0.2	0.21	208	0.81 ± 0.34	0.16	191	-0.08 ± 0.16	0.28
C/T + T/T	339	-11.0±0.2		324	-0.7± 3.0		251	0.07 ± 0.38		231	-0.32 ± 0.15	
CRP (rs1800947)												
C/C	573	-11.1±0.2	0.46	545	-0.9±0.1	0.21	410	0.43 ± 0.27	0.73	376	-0.14 ± 0.12	0.04
G/C + G/G	71	-10.8±0.4		67	-0.4±0.3		49	0.14 ± 0.93		46	-0.85 ± 0.33	
TNFA (rs1800629)												
G/A + A/A	169	-11.0±0.3	0.82	162	-0.6±0.2	0.17	117	0.94 ± 0.48	0.23	107	-0.32 ± 0.18	0.58
G/G	475	-11.1±0.2		450	-0.9±0.1		342	0.22 ± 0.31		315	-0.18 ± 0.13	
TNFA (rs361525)												
G/A + A/A	71	-10.6±0.4	0.24	65	-1.0±0.4	0.59	48	1.00 ± 0.71	0.43	43	-0.09 ± 0.29	0.71
G/G	573	-11.2±0.2		547	-0.8±0.1		411	0.33 ± 0.28		379	-0.23 ± 0.12	
IL6 (rs1800795)												
C/C	112	-11.0±3.7	0.88	102	-0.5±3.0	0.25	83	0.3±7.3	0.72	75	-0.2±2.0	0.84
G/C+G/G	532	-11.1± 3.6		510	-0.9±2.8		370	0.5±5.0		345	-0.1±2.4	

*ANCOVA analysis

The influence of variations in the pro-inflammatory genes on the correlation between changes in hs-CRP and changes in body weight during the weight maintenance period is shown in Table 7.4. Variations in *CRP* (rs1205 and rs1800947), *TNFA* (rs1800629), and *IL6* (rs1800795) genes influenced the correlations between changes in hs-CRP and body weight during the weight maintenance period in the whole group of subjects. Larger increases in hs-CRP were correlated with larger increases in body weight among the T-allele carriers of *CRP* rs1205 ($\beta= 0.190$, $p= 0.005$), CC genotype of *CRP* rs1800947 ($\beta= 0.174$, $p= 0.001$), GG genotype of *TNFA* rs1800629 ($\beta= 0.175$, $p= 0.003$) and G-allele carriers of *IL6* rs1800795 ($\beta= 0.206$, $p<0.001$). This correlation was not significant for the other genotypes of these genes. We found no influence of *TNFA* rs361525 on the correlation between hs-CRP and body weight changes.

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Table 7.4. Correlation between changes in hs-CRP and body weight during the weight maintenance period according to gene variations in inflammatory genes

Gene variations	Genotypes	n	Beta [^]	p
All subjects	N/A	420	0.164	<0.001
<i>CRP</i> rs1205	C/C	188	0.138	0.061
	C/T + T/T	232	0.175	0.007
<i>CRP</i> rs1800947	C/C	372	0.179	<0.001
	G/C+G/G	48	0.066	0.651
<i>TNFA</i> rs1800629	G/G	312	0.175	0.002
	G/A+A/A	108	0.118	0.222
<i>TNFA</i> rs361525	G/G	379	0.147	0.004
	G/A+A/A	41	0.368	0.021
<i>IL6</i> rs1800795	C/C	75	-0.002	0.985
	G/C+G/G	345	0.211	<0.001

[^]Linear regression test with correction for age, gender and study center

Variations in pro-inflammatory genes also might influence the association between self-reported protein intake and dietary GI and weight changes during the weight maintenance phase. In Table 7.5 it is shown that the positive correlation between GI and changes in body weight during weight maintenance was significant among those with the GG genotype ($p=0.044$) of *TNFA* rs1800629, but not in A-allele carriers ($p=0.487$) or any of the other genotypes. Percent protein intake was negatively correlated with changes of body weight only among those with the CC genotype of *CRP* rs1205 ($p=0.016$), CC genotype of *CRP* rs1800947 ($p=0.028$) and the G-allele carriers of *IL6* rs1800795 ($p=0.004$). Among those with other genotypes percent protein intake was not correlated with the change in body weight (all $p>0.05$). There was no influence of the gene variations in *CRP*, *TNFA* and *IL6* on the interaction between GI or protein intake with changes in hs-CRP (Supplementary Table 7.2).

Table 7.5. Correlation between GI or protein intake (% of total energy intake) with changes in body weight during the weight maintenance period based on gene variations in inflammatory genes.

	All subjects		<i>CRP</i> rs1205		<i>CRP</i> rs1800947		<i>TNFA</i> rs1800629		<i>TNFA</i> rs361525		<i>IL6</i> rs1800795	
	(n=453)		C/C (n=204)		C/C (n=404)		G/G (n=336)		G/G (n=407)		C/C (n=83)	
	Beta	p	Beta	p	Beta	p	Beta	p	Beta	p	Beta	p
GI [^]	0.080	0.148	-0.006	0.945	0.073	0.217	0.130	0.044	0.059	0.310	0.249	0.073
% protein*	-0.106	0.045	-0.198	0.016	-0.125	0.028	-0.115	0.064	-0.096	0.084	0.085	0.498
			C/T+T/T (n=249)		G/C+G/G (n=49)		G/A+A/A (n=117)		G/A+A/A (n=46)		G/C+G/G (n=370)	
	Beta	p	Beta	p	Beta	p	Beta	p	Beta	p	Beta	p
	GI [^]	0.124	0.103	0.280	0.128	-0.081	0.487	0.348	0.122	0.063	0.308	
% protein*	-0.034	0.625	0.026	0.869	-0.058	0.587	-0.113	0.567	-0.172	0.004		

[^]Linear regression test with correction for gender, center, BMI during randomization, protein and energy intake (mean week 4 and week 26 of weight maintenance phase); *Linear regression test with correction for gender, center, BMI during randomization, and GI (mean week 4 and week 26 of weight maintenance phase).

DISCUSSION

Inflammation has been suggested to play a role in weight regain following weight loss. Therefore we hypothesized that variations in inflammatory genes could influence variations in weight regain after weight loss among individuals with overweight/obesity. In this study, we showed that variation in inflammatory genes was not directly associated with body weight at baseline and with weight changes during the weight loss or the weight maintenance period. However, variations in inflammatory genes influenced the interaction between diet and weight regain.

We found that individuals with the CC genotypes of the *CRP* gene (rs1205 and rs1800947) had higher hs-CRP levels at baseline. This result is in line with an observational study in Iceland [27] that showed that individuals with the dominant genotype of *CRP* rs1205 had higher hs-CRP levels than those with other genotypes. Rs1205 shows after gene expression as a G>A polymorphisms in the CRP mRNA, which is located at the 3' untranslated region. Therefore, this region could be involved in the regulation of the RNA transcription process, and may affect RNA stability as well as translation and localization [28]. It has been suggested that RNA produced by the A allele is less stable than that produced by the dominant G allele and this could explain the higher hs-CRP concentration in CC genotype of rs1205 and GG genotype of rs1800947) [27,29]. Hage et al [14] reviewed the effect of variation occurring at several locations in the *CRP* gene, rs1205 and rs1800947, among others, on hs-CRP concentration. Based on findings from the literature, they suggested that the C allele of rs1205 and the G allele of rs1800947 were associated with higher hs-CRP concentrations [14]. These patterns were also seen in this study at baseline.

Besides findings showing that obesity is associated with increased inflammation, it is also suggested that inflammation plays a role in development of obesity. This theory is supported by reports showing that variations in proinflammatory genes are associated with the risk of obesity [13,14, 30-32]. However, to our knowledge there are no previous reports indicating that variations in pro-inflammatory genes are associated with weight loss or weight regain after weight loss. We found no direct association between gene variations in *CRP*, *TNFA* and *IL6* and body weight at baseline, weight loss and weight regain. However, we showed that the correlation between changes in hs-CRP and weight regain was influenced by variation in inflammatory genes. In those with the CRP increasing allele genotypes of the *CRP* gene, CC for rs1205 and G/C+G/G for rs1800947, changes in hs-CRP were not correlated with changes in body weight.

Variations in the *TNFA* gene might influence the correlation between GI and weight regain. In contrast to the report of the full DiOGenes trial where participants randomized to the higher GI groups regained more weight than those in the low GI groups (Larsen et al [12]), we found that a higher GI of the diet, based on self-report by means of dietary records, during weight maintenance was not correlated with more weight regain. This might due to several factors, including the use of less reliable self-report of the GI of diet, the observational analysis, as well as smaller

number of subjects in the whole analysis (not all subjects filled in the dietary records and not all were genotyped). However, the genotype of *TNFA* rs1800629 differentially affected this correlation. Interestingly, this genotype was also the one that influenced the correlation between changes in hs-CRP and weight regain. This might indicate that the influence of GI of the diet on weight regain is modulated by TNF-alpha concentrations. It was previously reported that a higher GI was associated with weight regain via up-regulation of inflammation markers, including TNF-alpha [33]. Unfortunately, TNF-alpha concentrations were not measured in the DiOGenes trial.

We found a negative correlation between percent protein intake during weight maintenance and body weight regain, as in the full DiOGenes trial (Larsen et al [12]). Gene variations at *CRP* (rs1205 and rs1800947), and *IL6* (rs1800795) influenced this correlation. Among those with the CC genotype of *CRP* rs1205, CC genotype of *CRP* rs1800947 and the GC+GG genotype of *IL6* rs1800795, percent protein intake was significantly negatively correlated with weight regain, in the other genotypes the negative correlations did not reach statistical significance. A recent publication from the longitudinal Framingham Heart Study Offspring Cohort [34]. reported that a higher dietary protein intake was associated with a smaller increase in inflammation. By contrast, in the DiOGenes study a high protein diet was not associated with changes in hs-CRP [4]. To our knowledge, this is the first study that observes a role for variations in pro-inflammatory genes in the correlation between protein intake and weight regain following weight loss. Further studies are needed to evaluate the mechanism behind this effect.

There were several limitations to this study. Firstly, although the DiOGenes trial was a randomized controlled trial with 5 different dietary groups, the reported analysis was done by merging all data and perform the analysis as an observational study. Subjects were only separated based on genotype. This was due to the small number of subjects when subjects were divided into diet x genotype groups. Secondly, dietary intake was self-reported using food records which might influence the accuracy of data. Lastly, the number of subjects in a specific genotype group was limited. It is recommended for an observational study to have at least 500 subjects per effect allele [35]. Thus, further study with a larger sample size is needed to confirm the results of this analysis.

In summary, this study provides some preliminary evidence for the influence of variations in inflammatory genes on the interaction between dietary intake, inflammation and weight regain following weight loss. This finding supports the previous notion that inflammation plays a role in weight regain following weight loss. Gene variations in *CRP*, *TNFA* and *IL6* genes were not associated with body weight at baseline, weight loss and weight regain. However, these gene variations influenced the correlation between protein intake and GI of the diet and weight regain following weight loss. Further study is needed to evaluate mechanisms on how protein and GI of the diet and variation of inflammatory genes interact.

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Supplementary Table 7.1. Allelic distribution and HWE of all genotypes.

Gene	Rs number	Major Allele	Minor Allele	HWE p
<i>CRP</i>	RS1205	C (n=1043)	T (n=495)	0.56
<i>CRP</i>	RS1800947	C (n=1450)	G (n=88)	1.0
<i>TNFA</i>	rs1800629	G (n=1306)	A (n=232)	0.011
<i>TNFA</i>	rs361525	G (n=1452)	A (n=86)	1.0
<i>IL6</i>	rs1800795	G (n=929)	C (n=609)	0.083

Supplementary Table 7.2. Correlation between GI and protein intake (% of total energy intake) with changes in hs-CRP concentration during the weight maintenance period based on variations in pro-inflammatory genes.

	All subjects (n=0.420)		<i>CRP</i> rs1205 C/C (n=204)		<i>CRP</i> rs1800947 C/C (n=404)		<i>TNFA</i> rs1800629 G/G (n=336)		<i>TNFA</i> rs361525 G/G (n=407)		<i>IL6</i> rs1800795 C/C (n=83)	
	β	<i>p</i>	β	<i>p</i>	β	<i>p</i>	β	<i>p</i>	β	<i>p</i>	β	<i>p</i>
GI [^]	0.099	0.100	0.028	0.723	0.069	0.240	0.019	0.768	0.027	0.633	0.007	0.963
% protein*	-0.005	0.929	-0.066	0.389	-0.003	0.962	-0.029	0.621	-0.010	0.855	0.159	0.218

	C/T+T/T (n=249)		G/C+G/G (n=49)		G/A+A/A (n=117)		G/A+A/A (n=46)		G/C+G/G (n=370)	
	β	<i>p</i>	β	<i>p</i>	β	<i>p</i>	β	<i>p</i>	β	<i>p</i>
GI [^]	0.054	0.482	-0.109	0.564	0.121	0.296	0.216	0.268	0.842	0.400
% protein*	0.045	0.517	-0.020	0.903	-0.047	0.635	0.059	0.733	-0.029	0.612

[^]Linear regression test with correction for age, sex, center, BMI during randomization, protein and energy intake (DI mean week 4 – week 26); *Linear regression test with correction for age, sex, center, BMI during randomization, and GI (DI mean week 4 – week 26).

CHAPTER 8

General Discussion



Inflammation might play a part in body weight regulation. Previous studies showed that circulating inflammatory markers [1,2], inflammatory properties of the diet [3,4], and genetic variation in inflammatory markers [5-7] are associated with obesity. To date, it is not known whether the interaction between inflammation and obesity could also be targeted in the context of weight change during and after a weight loss program and whether diet might influence this association.

The general objective of this thesis was to evaluate the influence of diet and inflammatory gene variations on inflammation and body weight changes in obesity. The objective was specified into 4 aims: 1) To evaluate the association of the inflammatory properties of the diet with weight regain after a weight loss program as well as with the risk for obesity in a population-based study; 2) To develop a new low calorie dietary regime with low inflammatory property for weight loss and evaluate the association between the inflammatory properties of the diet and weight regain; 3) To examine the influence of inflammatory gene variations on the association between obesity and inflammation; 4) To evaluate the interaction between inflammatory gene variations and diet composition and its effect on weight regain after weight loss.

THE EFFECT OF DIET ON INFLAMMATION AND BODY WEIGHT: AN OBSERVATIONAL ANALYSIS

The habitual dietary pattern affects circulating inflammatory markers in adults [8]. The Dietary Inflammatory Index (DII) has been developed to assess the inflammatory properties of the diet by calculation of intake of nutrient and non-nutrient components of the diet [9]. The score on this index has been shown to be associated with systemic inflammation [10–12] as well as weight gain and obesity [13,14].

We investigated the association between DII, adiposity, and leptin concentration among Indonesian adults in a cross-sectional manner. As presented in Chapter 2 we found that the DII score was not correlated with any of the adiposity measures. Several reasons might explain this negative result. First, there was a lack of DII components compared to the Global Diet (as reported by Shivappa et al) due to limited nutrients data on found abundantly in Indonesian foods. Second, there could be some data quality issues including accuracy of dietary assessment using a semi-quantitative food frequency questionnaire. Lastly, other factors that were not measured in this study also contribute to body mass index and obesity status.

Interestingly, we showed that the DII was positively correlated with plasma leptin concentration after correction for age, sex and BMI, energy intake, and physical activity. Leptin is produced by adipocytes with an ability to induce production of inflammation markers such as which TNF-alpha and IL-6 [15,16]. The concentration of leptin increases as adiposity increases [17] and leptin has been proposed as a key link between obesity, inflammation and metabolic diseases. This is supported by several studies, which showed that a higher leptin concentration was associated with increased risk for cardiovascular diseases [18,19]. Although in our cross-sectional

study we showed that DII was not associated with adiposity, the positive correlation between DII and leptin indicated that there may be a connection between diet and inflammation, which might impose a risk of weight gain and/or cardiovascular diseases later in life.

We further investigated the effect of the inflammatory properties of diet on weight regain following weight loss among Dutch adults with obesity (Chapter 3). In this study we showed that the DII was positively correlated with weight regain, suggesting that inflammation may play a role in the regulation of body weight after weight loss. Additionally, we also showed that the intake of several individual micronutrients with anti-inflammatory properties, such as magnesium, folate, and riboflavin, were negatively correlated with weight regain. However, results from this study should be interpreted with caution. Since the analysis was done in an observational manner, no cause-effect relationship can be derived from this correlation.

Results from those 2 studies support the notion that inflammatory properties of diet might have a role in weight regulation, and possibly through regulation of inflammatory markers in the circulation as well as in adipose tissue (as marked by leptin concentration). In addition, results from these studies provide a practical implication to reduce the inflammatory properties of diet among individuals in the study population. This includes several key components of DII which influence weight gain such as magnesium, folate, and riboflavin. Those nutrients are rich in vegetables, nuts, and seeds.

The information generated at Chapter 2 and Chapter 3 provides some evidence that diet can influence inflammation as well as weight gain after weight loss, which might be interconnected. Those studies also provide us with key components to focus on regarding dietary modifications which might influence inflammation which includes increasing intake of fruits and vegetables, white meat, and legumes and reduction of red meat and processed foods. To test the hypothesis that the inflammatory properties of the diet play a role in weight management, a new dietary regimen for weight loss needs to be developed to reduce the inflammatory properties of the diet and thus influence the inflammatory response to weight loss, which might also be beneficial to prevent weight regain after weight loss.

THE EFFECT OF DIET ON INFLAMMATION AND BODY WEIGHT: AN INTERVENTION STUDY

To further investigate whether the inflammatory markers had an influence on weight loss, we developed a new low-calorie diet, called low calorie low inflammatory diet (LCID). The main objective of the study presented in Chapter 4 was to investigate the effect of weight loss on different inflammatory markers and whether this effect is modifiable by diet (LCID) in adults with obesity. We hypothesized that when reduction of inflammation by dietary modification can have influence on weight loss, this can prove that indeed, inflammation affects weight loss.

We found that after 8 weeks of the weight loss program there was an increase in plasma TNF-alpha and IL-6 concentrations. Subjects in the LCID group had a greater reduction in hs-CRP at the end of the intervention compared to those in the conventional low-calorie diet (LCD) group, but no differences were seen in changes of TNF-alpha and IL-6 concentrations between groups. The LCID induced reductions in body weight, body fat, and metabolic syndrome parameters without significant difference compared to LCD.

The controversies around the influence of weight loss on changes in inflammation markers have been reported before [20-27]. We showed that the hs-CRP concentration was on average not affected in the total group, but that it was reduced in the LCID group compared to the LCD group. By contrast, no group difference was found for the changes in TNF-alpha and IL-6. TNF-alpha and IL-6 are markers of adipose tissue inflammation while hs-CRP is a marker of liver inflammation [28,29]. Thus, it is speculated that the weight loss-induced increase in the inflammatory status of the adipose tissue is not sensitive to short term changes in the inflammatory properties of the diet. By contrast, the liver remains responsive to the dietary composition under the conditions of energy restriction.

The study in chapter 4 also shows that there is a challenge in optimizing the compliance to a weight loss diet that is being delivered to Indonesian adults with obesity. The adherence to the LCD and LCID regime was relatively low and this resulted in a small weight loss. During the personal meetings with the nutritionist, dietary recommendations were given by explaining the principles of the diet and providing a one-day meal plan that could be followed by the participant. Moreover, different from LCD which focused solely on reducing energy intake, LCID focused in addition on changing the dietary pattern. Crucial components such as eating vegetables in every main meal, increasing fish intake, and using oil with less saturated fat (i.e. olive oil) are hard to achieve in Indonesia and there was a significant variability in compliance among subjects. Despite the fact that 2-weekly meetings with nutritionists were included in the intervention, further effort is needed to improve compliance to prescribed dietary intake by incorporating behaviour change techniques.

The DII was used to estimate the inflammatory property of the diet in chapters 2 and 3, but no longer used in the subsequent chapters. There are several reasons for this. First, the DII was developed for use in observational studies and relies on self-reported data. This technique is known to have a questionable validity and reliability for measurement of dietary intake [30]. Second, the DII is highly dependent on energy intake. As chapter 4 evaluated the impact of calorie-restricted diets, the DII values changed and became incoherent. Third, there was a lack of DII components compared to the Global Diet due to limited nutrient data for Indonesian foods. For instance, we did not have complete data of several fatty acids (i.e. omega 3 and omega 6) in some Indonesian foods. This might influence the accuracy of DII calculation. Lastly, there are some unsettled controversies whether certain nutrients

are pro-inflammatory or anti-inflammatory. In the DII list, vitamin B12 is regarded as proinflammatory but several studies have reported that vitamin B12 is conversely associated with inflammation [31,32]. In addition, DII acknowledges alcohol as an anti-inflammatory component, but the influence of alcohol intake on inflammation is controversial. While a moderate intake of alcohol is associated with a reduction of inflammation [33,34], heavy alcohol intake is associated with increased inflammation [34]. This suggests that the correlation between alcohol intake and inflammation is U-shaped rather than linear, as assumed in DII.

The impact of LCID on weight loss and inflammation markers was evaluated and presented in Chapter 4. Despite the reduction of body weight due to dietary modification, changes in dietary intake were not strong enough to influence weight regain after weight loss. This analysis suffered from loss to follow up. This was due to several issues including: 1) loss of engagement in study participants, nutritionists and the research team; 2) follow-up was not pre-planned: the study was offered as a weight loss program, thus participants lost their interest once weight loss was achieved; 3) no direct financial incentive for participants after finishing the weight loss protocol; 4) some participants had a hard time reducing weight, so they hesitated to be remeasured. Innovations to increase motivation of study participants during a weight loss program and weight maintenance are necessary. The use of technology such as social media or mobile applications might help tackle this issue. Creating a supportive community might be necessary to prevent drop-out and improve engagement of study participants.

THE INFLUENCE OF DIET ON GENOMIC HEALTH

In addition, to evaluate the influence of LCD or LCID on inflammation markers and weight changes during weight loss, we also evaluated whether LCD or LCID were associated with markers of genomic health such as relative telomere length (RTL) and mitochondrial DNA copy number (mtDNA-CN). The reason for this analysis was that both RTL and mtDNA-CN are altered by chronic inflammation [35-37]. Increased inflammation induces shortening of RTL and reduction of mtDNA-CN [35-38] which are regarded as markers of aging. Interestingly, these markers of genomic health are associated with weight gain in adults [39]. In this study (Chapter 5), we showed that weight loss was associated with elongation of RTL but had no influence on mtDNA-CN. In addition, we also showed that those in the LCID group had a greater elongation of RTL compared to those in the LCD group. This analysis showed that modification of dietary composition during a weight loss program could improve health beyond inflammation, by improvement of genomic health. Since telomere shortening may predispose individuals to age-related diseases and increased mortality, the slowing down of this process by diet can help protect from degenerative diseases and improve wellbeing.

THE INFLUENCE OF INFLAMMATORY GENE VARIATIONS ON THE INTERACTION BETWEEN BODY WEIGHT AND INFLAMMATION

Low-grade systemic inflammation is induced by several factors including environmental exposures (diet, physical activity, pollutants) and genetic background [38]. There is evidence that variants of inflammation-related genes influence the interactions between adiposity and inflammation. For example, Eiriksdottir et al. [40] reported that variants of the *CRP* gene modulate the relationship between body mass index and high-sensitive C reactive protein (hs-CRP). This was supported by other studies [41,42] showing the role of variations at the *CRP* gene on the interaction between obesity and hs-CRP. The objective of Chapter 6 was to investigate associations between SNPs at *TNFA*, *CRP*, and *IL6* and measures of adiposity, as well as the correlation between adiposity and hs-CRP among adults living in Northeast England.

In this analysis we report that variation in *TNFA* (rs1800629) was associated with adiposity, but variations in *CRP* (rs1800947, rs1205) and *IL6* (rs1800795) were not. We also reported that variation in pro-inflammatory genes plays a role in the interaction between adiposity measures and hs-CRP. Among subjects with the GG genotype of *TNFA* (rs1800629), CG+GG genotype of *IL6* (rs1800795), GG genotype in *CRP* (rs1800947) and CT+TT genotype of *CRP* (rs1205), the correlation between adiposity measures and hs-CRP was significant.

To further confirm the influence of inflammatory gene variations on the correlation between hs-CRP and body weight, an analysis was performed among individuals who undertook a weight loss program in the DiOGenes Study (Chapter 7). Similarly, we evaluated the influence of gene variations in *TNFA*, *CRP* and *IL6* on the correlation between changes in body weight and hs-CRP concentration during the weight maintenance period. In this study, we showed that among those with the CT+TT genotype of *CRP* (rs1205), CC genotype of *CRP* (rs1800947), GG genotype of *TNFA* (rs1800629), and CG+GG genotype of *IL6* (rs1800795) changes in body weight were positively correlated with hs-CRP during the weight maintenance period. These data confirm the observational study in Northeast England which showed that variations in *CRP*, *TNFA* and *IL6* genes influence the correlation between body weight and hs-CRP.

THE INFLUENCE OF INFLAMMATORY GENE VARIATIONS ON THE INTERACTION BETWEEN DIET AND WEIGHT REGAIN FOLLOWING WEIGHT LOSS

In Chapter 7, we investigated the interaction between inflammatory gene variations, diet and weight gain after weight loss in DiOGenes Study. This was to examine the hypothesis that inflammatory genes play a role in the regulation of body weight by affecting the correlation between diet and weight gain. In this study we showed that variations in inflammation genes were not directly associated with body weight at

baseline and with weight changes during the weight loss or the weight maintenance period. However, these variations influenced the interaction between diet and weight regain. Among those with the CC genotype of *CRP* rs1205, CC genotype of *CRP* rs1800947, and the GC+GG genotype of *IL6* rs1800795, percent protein intake was negatively correlated with weight regain, in the other genotypes the negative correlations did not reach statistical significance. In contrast, the correlation between GI and changes in body weight during weight maintenance was significant among those with the GG genotype of *TNFA* rs1800629 but not in those with the GA+AA genotype. These findings show that the individual weight responses to a specific diet composition differ and that variations in inflammation genes might be involved in this variation. Findings from this study could, if replicated, perhaps be used in a clinical setting to improve dietary treatment by matching with an individual's genetic background. By matching dietary recommendations with individual genetic background, we could personalize dietary advice to optimise results.

STUDY LIMITATIONS

There were several limitations in this study. First, the analysis of the influence of inflammatory properties of diet on adiposity and weight gain was done as an observational study. Thus, no cause-effect relation could be derived from those studies. Second, an effort to reduce inflammation by dietary modification was done in an intervention study. However, such an attempt has limitations due to a relatively short research period, small differences in dietary patterns between intervention and control groups, as well as the difficulty to comply with the LCID regime. Lastly, analysis on the influence of inflammatory genes variation on adiposity and weight gain was done in an observational manner.

Further studies are required to clarify findings from this study. A randomized controlled trial with a sufficient number of subjects is needed to test whether variation on inflammatory genes could influence the effect of high protein/GI or low protein diet/GI on weight regain. In addition, a longer intervention duration is needed to evaluate the impact of LCID during a low-calorie diet intervention and its follow-up. Compliance with dietary recommendations is an issue and this might be so because of the current model of dietary practice in Indonesia, where clients receive all dietary instructions at the same time and have to integrate them with their daily lifestyle. The future dietary intervention might take benefit from mobile applications that integrate daily recommendations with virtual lifestyle coaching. By using mobile technology, the dietary recommendations can be delivered one at a time with a pace adjusted by clients, not the other way around. In addition, this technology also allows more personalized and real-time consultation as problems with diet occur.

All in all, the data presented in this thesis support the hypothesis that inflammation plays a role in the regulation of body weight. We showed that dietary factors associated with inflammation could influence weight regain following weight loss. We also reported that pro-inflammatory gene variation was associated with body weight. In this thesis, we indicated that there was an interaction between diet and gene on the association between inflammation and body weight.

CONCLUSION

In summary, we showed that the inflammation property of the diet is associated with adipose tissue inflammation, marked by leptin concentration, and partially explains variations in weight regain following weight loss. The modification of a low-calorie diet to reduce inflammation (LCID) in the short term lowers hs-CRP, but does not influence changes in TNF-alpha, IL-6, or weight loss. The LCID was also not associated with weight regain after weight loss. However, the LCID had a positive impact on improving genomic health by elongation of RTL. We showed that gene variations in inflammatory genes such as *CRP*, *TNFA*, and *IL6* influenced the correlation between obesity and inflammation. Variation in these genes also influenced the correlation between diet composition, e.g. protein and glycemic index of diet, and weight regain following weight loss.

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THE INTERACTION

BETWEEN INFLAMMATORY
PROPERTIES OF DIET AND
GENETIC VARIATION IN

BODY WEIGHT REGULATION

IMPACT

A low-calorie diet has been a long-standing practice among nutritionists and dieticians as a core component of a weight loss program. The concept is when our diet provides less energy than needed, our body will start to break down fat to fulfill the energy demand. With regards to calorie counting, several methods have been developed in several decades such as calorie restriction (reducing 300 kcal/day), a low-calorie diet (1500 kcal/day), and a very low-calorie diet (800 kcal/day). However, these methods also showed that the pitfall of an energy deficit diet is the tendency to weight regain after weight loss is achieved.

Study Objectives and Conclusion

Scientific findings in recent years have indicated that there are several mechanisms that lead to weight regain, one of them is inflammation. Inflammation is a signal produced by cells in our body that has an influence on the behaviour of other cells, including immune cells. Traditionally, inflammation is seen as immune reaction to pathogen infection. However, in recent years it has become evident that inflammation also has an influence on other elements of human physiology including energy and substrate metabolism. The impact of inflammation on metabolism might explain its role in the mechanism of weight regain following weight loss.

The main objective of this research is to investigate whether inflammation plays a role in body weight regulation based on population and intervention studies. In this thesis, we support other evidence that inflammation plays a role in body weight regulation. Inflammation can be triggered by several factors including diet and genetics. In a weight loss program conducted in The Netherlands, we showed that when dietary intake was more likely to increase inflammation, individuals were more likely to gain weight following weight loss. We further created a new dietary regime for weight loss with the aim not only to reduce calorie intake but also to reduce inflammation, a low-calorie-low inflammatory diet (LCID). Although we showed that the new diet may reduce inflammation over a short period of time, this diet did not significantly improve body weight loss compared to the conventional low-calorie diet.

Because diet is not the only factor that triggers inflammation, we further evaluated whether genetic variation might play a role in the interaction between diet and weight gain. In this study, we showed that the individual response to a diet was influenced by variations in selected pro-inflammatory genes. These genes encode proteins that are direct pro-inflammatory signals. The weight regain response to a high protein diet or a low glycemic index diet, which were previously shown to reduce weight regain, was different according to genetic variation.

Potential Scientific Contribution

There are several main messages to society that can be obtained by the studies in this dissertation. First, dietary modification to reduce calorie intake and inflammation is possible by not only focusing on reduction of the amount of food consumed but also on improved dietary habits. This can be done by selecting foods that are high in nutrients such as vitamins (vitamin A, B, C, and E) and minerals (magnesium, zinc, selenium), which are anti-inflammatory, and reducing consumption of foods that have pro-inflammatory properties (such as energy-dense, high saturated and trans fat foods). Such efforts have been successfully reported in the Mediterranean diet.

Second, if reproducible, this finding can be further developed into a personalized nutrition approach for weight management. Despite controversies among academia, dietary recommendations based on gene variations are currently being developed in real-world setting by several tech start-ups. There are “personalized nutrition” services that integrate data of gene variations in dietetic consultation or nutritional services. International tech company such as Nutrigenomix, DNAfit, DNAudge, and Gini have successfully developed their own nutrigenetic approach for personalized nutrition. The rise of direct to customer genetic testing, such as 23andme, may give personalized nutrition a place in the future of dietetics. In Indonesia, there are 2 providers of personalized nutrition services based on genetic variation, namely Kalbe Nutrigen-me and Prodia Nutrigenomics. While the services are available, scientific studies are needed to expand our current knowledge on gene-diet interaction as well as providing accurate information regarding dietary recommendations based on genetic profiles.

The “personalized nutrition” approach using genetic information raises some social challenges. It has been criticized that current marketing strategies seem to overpromise the result of personalized nutrition to their clients. On the other side, the area of service seems to lack regulation at the national and international level. Further action is needed by governments, academics and industry in the regulation and commercialization of personalized nutrition.

Target Audience of The Scientific Findings

Obesity is global health problem that costs millions of dollars for treatment as well as treatment of diseases related to obesity. This research aimed to contribute to alleviating this problem by development of a weight loss program using a different approach, by dietary modification and genetic analysis. Results from this study can be used by those who work in the health sector as well as wellness industry. This study focused on one of the most challenging problems facing obesity treatment, namely the weight regain following weight loss.

Dietitians and nutritionists are two major target audiences for this study. We provided information on modification of the diet during a weight loss program. This included the role of inflammation on weight regain and how an individual can

response differently due to its genetic background. This information is aimed to educate practitioners in the health and wellness industry to not only focus on reducing calorie intake but also to improve the diet quality. Fellow scientists working in the areas of nutrition and nutrigenetics all around the world are also target audience of this study. It is hoped that results from this study can be used as a basis for the development of methods to reduce inflammation during a weight loss program.

Activities for Result Dissemination

The main outlet of this research is the scientific publication of the studies that are currently done within this thesis. This will provide scientific insight on how the studies were conducted and conclusions that were generated and their strengths and limitations. Results from this study has been presented at several conferences including *Asia Pacific Nutrigenomic and Nutrigenetic Conference* (China, 2020), *NuGO Week* (United Kingdom, 2018), *Dutch Nutrition Science Day* (The Netherlands, 2018), *European Conference on Obesity* (Austria, 2018). I also wrote a book with title “Diet Mediterania: Teori dan Aplikasi Bagi Masyarakat Indonesia” or “Mediterranean diet: Theory and Application for Indonesians”. The book is a guide on how to adapt Mediterranean diet principle to Indonesians. The Mediterranean diet itself has been an important insight with respect to the dietary approach to reduce inflammation which inspired the development of the low-calorie-low-inflammatory diet.

In addition to the book and journals, I also plan to create a 10 minutes education video for 3 topics : 1) the influence of dietary habits on inflammation; 2) the importance of keeping inflammatory low when dieting; and 3) individual response to diet based on gene variations. The video will be an animated version of this thesis and made in English and will be broadcasted my YouTube page “Gizi Gama”. By doing this, I can provide information from this research freely to the public, practitioners in the health and wellness industry, as well as fellow academics.

ACKNOWLEDGEMENTS

To work in the scientific field, having a Ph.D. degree is the basic requirement. And after 4 years of undergoing the educational system, I now understand why it is important. Studying in a doctoral school teaches us how to understand the phenomenon and obtaining knowledge from its own source. And being a Maastricht University Ph.D. student gave me a tremendous experience not only to how to understand how to conduct research better, but also present it in a paper and presentation, and have a collaboration with fellow scientists in the field.

This dissertation is written by a Ph.D. student with an external Ph.D. scheme which means I do not have a full-term scholarship or fixed research grant to support what I did in the last 4 years. Thus, this program cannot be happening without “tons” of supports from individuals and organizations that cross this path.

I would like to express my gratitude to Prof Marleen van Baak for the help, support, and guidance through 4 years of Ph.D. activities. Its is a humbling experience working with human experiment conducting a lifestyle modification program. Her experiences working in these fields really help me a lot in understanding the context of what I did. I also would like to thank Prof. Edwin Mariman for his help during the Ph.D. program. Help me guided my research from the very beginning on the molecular aspect of obesity. Thank you for believing me, especially that I can finish this doctoral school on time.

Both of my promotors not only providing me with guidance but also showing me the way of research and development in this topic. And also to point out several things that are missing in the literature. What they did inspires me to keep working in this direction. I remembered in 2011, when I was a master’s student of Biomedical Sciences at Maastricht University, deciding to work in their lab. And 10 years later, I graduated from the same supervisor with a similar research topic. I surely would continue working in the area of genetic and obesity with an emphasis on weight loss.

Thank you Prof. dr. E.E. Blaak, Prof. dr. J. Plat, Prof. dr. A. Salis, Prof. Dr. R. Mensink, Dr. Marleen van Greevenbroek and Dr. ir. R. Stienstra for taking the time and effort to review this thesis and being present at the official Ph.D. defense.

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IMPACT & ACKNOWLEDGEMENTS

To Dr. Safarina, Wina, Sukma, and Asha from The Eijkman Institute, I would thank the opportunity of learning from your lab on molecular analysis as one of the topics in this study. Working at one of the prestigious Indonesian laboratories for molecular biology is really a moment of pride for me. When I was in high school, I used to dream of working there as a scientist. And those are the moment when dreams do come true.

Thank you, Prof Wan Manan and Dr. Yi Yi Lee, from Universiti Sains Malaysia (International Medical University) who in the early stage of this research supported me with the research collaboration. A study was initially conducted in 2016 is developing into a Ph.D. project. On this note, I also would like to thank Dr. Emy and Miss Dian for their collaboration and commitment to this project.

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I would thank several organizations that in the past few years helping me with financial supports so that I can finish my Ph.D. Badan Penerbit dan Publikasi Universitas Gadjah Mada, World Obesity Federation, The European Nutrigenomic Organization, and European Association for Study Obesity.

While studying at Maastricht University for a Ph.D., I met a significant amount of people that not only help me through the journey but also make this process memorable. To Adriyan, who I met for the first time in Maastricht that help me out through my journey and amazing moment with his family Fadhlán, Mima and Uchi. And the lunch people Manuel, Kenneth, Rens, Sultan, Lars, and Jihad, thank you for all the talks during lunch hours. You all will be missed. I thank Pak Fauzan and his family who help me while I am there. I feel like I found a home a way from home. Thank you Cleo and Claudia for helping me with anything needed during my stay at Maastricht University.

Going back and forth between Indonesia and The Netherlands is not always easy. Thus to friends in Maastricht that I know for a decade, Ivo, Birke, Monica, Dennie, Nico, and Thijs, thank you for making Maastricht feels like a second home to me. Thank you for all the dinner invitations and movie nights and the hangouts (or baby day out). Nuria, thank you for all the support and perspective on things in life and in nutrition-wise that you generously share.

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IMPACT & ACKNOWLEDGEMENTS

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Harry Freitag Luglio Muhammad was born on 11 July 1986 in Dili, Timor Leste. After the independence of Timor Leste, his family chooses to live in Indonesia. Here, Harry finished his high school and bachelor's degree in Java island. He received bachelor of Nutrition from Universitas Gadjah Mada, Yogyakarta, Indonesia in 2008. In 2009, he finished his dietetic internship program.

In 2011, he continue his study on Master of Biomedical Sciences at Maastricht University with a specialization in nutrition and metabolism. His thesis is under the supervision of Prof Marleen van Baak. After finishing his master's degree, he return to Indonesia in 2013. At the Department of Nutrition and Health, Universitas Gadjah Mada, he teaches bachelor students of nutrition as well as to conducts scientific studies. Since then until recently, he published around 40 publications in national and international peer-reviewed journals, 5 academic books, and 1 book chapter.

November 2017 is when he started his Ph.D. at Maastricht University. Between 2017 and 2021, he received several grants and awards including Travel Grant from Badan Penerbit dan Publikasi, Universitas Gadjah Mada, Scope School Scholarship, World Obesity Federation, NuGO Students Exchange Grant, European Association for Study Obesity Travel grant. Those grants were necessary for his Ph.D. because this allowed him to conduct research as well as to travel within his Ph.D. program.

In addition to conducting research and teaching student, Harry is also involved in several organizations. Including Asia Pacific Nutrigenomic Nutrigenetic Organization, Nutrigenomic Early Career Scientist, and World Obesity Federation.

Harry is active on social media. He now has YouTube, Instagram, Twitter, Spotify and a website (gizigama.com), in which he routinely posts educational materials for the general public. He is also currently working with several start-ups and company including the Indonesian Nutrition and Fitness app FITA as a "Signature Coach" as well as Cordlife Genetic as an educator for direct-to-customer genetic tests for the general public and professionals.



LIST OF PUBLICATIONS

1. Muhammad HFL, Pahdarina D, Zahara NP, Nugraheni F, Hanny TA, Ermamilia A, Huriyati E. Diet or exercise: The role of diet and/or exercise on changes of pro-inflammatory markers during a weight loss program in adult women with overweight. **Clinical Nutrition ESPEN**, 2021, 44:337-341.
2. Muhammad HFL, Pramono A, Rahman NH. The safety and efficacy of supervised exercise on pregnant women with overweight/obesity: A systematic review and meta-analysis of randomized controlled trials. **Clinical Obesity**, 2021, 11(2):e12428.
3. Muhammad HFL, Sulistyoningrum DC, Huriyati E, Lee YY, Wan Muda WAM. The Interaction between Energy Intake, Physical Activity and UCP2 -866G/A Gene Variation on Weight Gain and Changes in Adiposity: An Indonesian Nutrigenetic Cohort (INDOGENIC). **British Journal of Nutrition**, 2021, 125(6), 611-617.
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10. Marliya, Muhammad HFL. Introducing The New Nutrition Guideline to Indonesian Overweight/Obese Adolescents Using A Short Movie: The Impact on Nutritional Knowledge, Eating Habit and Dietary Intake. **Progress in Nutrition**. 2019; 21 (Suppl 1):227-233.
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16. Susetyowati, Huriyati E, Kandarina BJI, Muhammad HFL, Faza F. Prevalence and determinants of high blood glucose in urban and rural Indonesian adult population. **International Journal of Diabetes in Developing Countries**, 2019, <https://doi.org/10.1007/s13410-019-00721-6>.
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ORAL PRESENTATIONS

1. The Effect of a Low Calorie Low Inflammatory Diet on Inflammation, Telomere Length and Mitochondrial DNA among Obese Adults (**eNUGOweek 2021 European Nutrigenomic Organization**, 6-8 September 2021, online)
2. The Effect of a Low Calorie Low Inflammatory Diet on Inflammation, Telomere Length and Mitochondrial DNA among Obese Adults (**Asia Pacific Nutrigenomic**

and **Nutrigenetic Conference** 2020, 1-2 Desember 2020, Online)

3. The Interaction Between Energy Intake, Physical Activity and -866G/A UCP2 Gene Variation on Weight Gain and Changes in Adiposity: An Indonesian Nutrigenetic Cohort (INDOGENIC) (**Asia Oceania Conference on Obesity**, 29 Agustus - 2 September 2019, Seoul, South Korea).
4. The Interaction Between Uncoupling Protein 2 Gene Variation and Inflammatory Properties of Diet on Blood Pressure in Adults (**NuGO Week**, 3-6 September 2018, Newcastle, United Kingdom).
5. Assessment of a low calorie low inflammatory diet for weight loss of adults with obesity. (at **Dutch Nutrition Science Day**, 11-12 Oktober 2018, Heeze, The Netherlands).
6. Nutrigenomics and Nutrigenetics Aspect of Obesity and Body Weight Management. (**Indonesian Nutrigenomics and Nutrigenetics Association meeting**, 18-19 Oktober 2017, Bali, Indonesia).
7. Unravel The Ramadan Fasting Yoyo Effect : Indonesian Prospective Study on Overweight/Obesity Individuals. (**World Obesity 14th Stock Conference**, 31 March 2017, Sydney, Australia).
8. Gene-Lifesyle Interaction and Individual Predisposition to Obesity: Untangle the Complexity. (**Asia Pacific Nutrigenomic and Nutrigenetic Conference** 2016, 5-6 December 2016, Gyeongju, South Korea).
9. The Role of Genetic Variation in TCF7L2 and KCNJ11, Dietary Intake and Physical Activity on Fasting Plasma Glucagon Like Peptide -1 in Male Adolescents. (**Annual Indonesian Society of Human Genetic Conference**, 26-28 November 2016, Semarang, Indonesia).
10. Gene-lifestyle interaction: the role of snps in UCP2 -866G/A and UCP3 -55C/tondietary intake and physical activity in indonesian obese female adolescents. (**6th International Symposium on Wellness Healthy Lifestyle and Nutrition**, 25-27 November 2015, Kota Bharu, Malaysia).



AWARDS

- 2015 : ERASMUS MUNDUS AREAS+ for visiting scholar at KU Leuven, Belgium
- 2015 : MPV awards International Symposium on Wellness Healthy Lifestyle and Nutrition
- 2016 : ILSI South East Asia Travel Grant
- 2017 : Australian Award Fellowship
- 2017 : Australia Indonesia Centre Travel Grant
- 2017 : Asia Oceania Association for Study Obesity Travel Grant
- 2018 : European Association for Study Obesity Travel Grant
- 2018 : NUGO student Exchange Grant
- 2019 : Scope School Scholarship from World Obesity Federation



BOOKS (BAHASA INDONESIA)

- 2018 : Imunologi gizi, *Gadjah Mada University Press*
- 2018 : Obesitas translasional: aspek klinis & molekuler dari kejadian obesitas, *Gadjah Mada University Press*
- 2020 : Pedoman *personalized nutrition advice* berbasis variasi genetik untuk pencegahan penyakit kardiovaskuler, *Gadjah Mada University Press*
- 2021 : Diet mediterania : teori dan aplikasi bagi masyarakat Indonesia, *Gadjah Mada University Press*
- 2021 : Buku ajar nutrigenomik dan nutrigenetik bagi mahasiswa gizi, *Gadjah Mada University Press*
- 2021 (in press)
: NUTRIGENETIK
: Rekomendasi Kebutuhan Energi dan Zat Gizi Makro Berbasis Genetik Untuk Layanan Personalized Nutrition



BOOK CHAPTER (ENGLISH)

- 2019 : *Muhammad HFL*, Dickinson KC. Nutrients, Energy Values and Health Impact of Conventional Beverages (Book chapter : NUTRIENTS IN BEVERAGES, edited by Grumezescu AM and Holban AM). **Woodhead Publishing (Elsevier): United Kingdom.**



SOCIAL MEDIA

- ▶ Youtube : Gizi Gama (around 1K subscribers and 300 educational videos, since May 2020)
- 🎧 Spotify : Gizi Hari Ini
- 📷 Instagram : Gizi_Gama
- 🐦 Twitter : @gizigama
- 🔗 Website : gizigama.com