Balanced Nutritional Diet Intervention on Body Composition Among Different FTO RS9939609 Gene in Obese Young Women In Bandung, Indonesia: A Randomised Controlled Trial

Update 10 October 2023

No. Register:	2	3	0	7	0	6	1	0	8	9

A. General information

1.	Principal Researcher (Name and title)	Putri Novitasari, S.Gz., M.Si	
	Skills/Specialization	Nutrition Science	
	Position/Position	IPB Doctoral Student (S3)	
	Tel. House: –	MOBILE PHONE: 08112227291	e-mail: putrinovitasarigizi@gmail.com
2.	Origin of Institution: Non-Unpad Doctoral Study Program in Nutritional Sciences IPB University		
	Tel.: (0251) 8622642	Fax:–	e-mail: pascasarjanagizi@apps.ipb.ac.id
	Sponsors (Individual/Private/National grant/International grant)	Individual	
	Clinical Monitor	Dr. Lucky Angkawijaya Roring, M.Pd., AIFO	
	Other Supervisors/Researchers: 1. Prof. Dr. Rimbawan 2. Prof. Dr. Ir. Hardinsyah, MS 3. Prof. Dr. Ir. Hadi Riyadi, MS		
3.	Research Title: The Effect of a Balanced Nutritional Diet on Cardiovascular Risk Factors Among Two Types of FTO Gene rs9939609 Obese Young Adult Women		
4.	Multicenter	☐Yes	Primary Research Center:
			Satellite Research Flashlight:
		√ No	
5.	Study √ Not cooperation ☐National cooperation ☐International, number of countries please specify: ☐Involving a foreign Chief Researcher (attach permission, Institutional MoU, Researcher visa)		
6.	Filled in if a foreign research leader is involved		

No.	Name, title, institution of the foreign Research Chair	Duties & Functions	Tel., Fax, HP, e-mail:
1.			
2.			
3.			

7.	Research Place (Mention the name of the hospital, treatment room, polyclinic, or other health service location): Screening of potential subjects, signing of the INFORMED CONSENT, collection of subject blood specimens, and data collection were carried out at the Health Services (Polyclinic), Universitas Pendidikan Indonesia Address: Jl. Dr. Setiabudhi No. 229, Isola, Sukasari, Bandung City The doctor responsible for examining the subject, taking samples and managing if any side effects occur: Dr Lucky Angkawijaya Roring, M.Pd., AIFO

	If necessary, collection of additional or incomplete data is carried out online/by telephone or at the subject's place if the subject permits. Blood specimen testing was carried out at the Molecular Genetics Laboratory, Faculty of Medicine, Padjajaran University.		
8.	Research Time Plan: ± 4 months	Starting: November 2023	Completed: February 2024
9.	Data Collection Time Starting: November 2023 to February 2024		
10.	Has This Protocol Been Submitted to Another Ethics Commission?		
	☐Yes If yes, attach a photocopy of the document!	☐Accepted ☐Rejected	
	√ No		

11. Allocation and Details of Research Funds (Human Resources, Consumables, etc.):

No.	Type of Spending	Component	Fee Required
1	Material	ATK	IDR 500,000
2	Material	Diet Procurement (20x3x30x45,000)	IDR 81,000,000
3	Material	Elisa Kit (IL-6; hs-CRP; adiponectin)	IDR 23,532,000
4	Material	DNA isolation kit (40x200,000)	IDR 8,000,000
5	Material	Primary (1x400,000)	IDR 400,000
6	Material	Primary Design Services (1x150,000)	IDR 150,000
7	Material	Subject Reward (40x35,000)	IDR 1,400,000
8	Data collection	FGD research preparation (5x105,000)	IDR 525,000
9	Data collection	Survey Officer's Honorarium (Screening: 40x8,000)	IDR 320,000
10	Data collection	Temporary Blood Glucose Test (40x18,000)	IDR 720,000
11	Data collection	Researcher Administration Honoraria	IDR 300,000
12	Data collection	Transport subject + officers (43x25,000x2)	IDR 2,150,000
13	Data collection	Data collection meetings (5x105,000)	IDR 525,000
14	Equipment Rental	Research Equipment (2x350,000)	IDR 700,000
15	Specimen Analysis	PCR Gene Amplification (42x100,000)	IDR 4,200,000
16	Specimen Analysis	Sequencing (42x350,000) + (42x10,000)	IDR 15,120,000
17	Specimen Analysis	Electrophoresis (42x50,000)	IDR 2,100,000
18	Specimen Analysis	Elisa Analysis (3x1,800,000)	IDR 5,400,000
19	Data analysis	Honor Data Processor	IDR 1,540,000
20	Data analysis	Meeting Consumption Costs (5x105,000)	IDR 525,000
21	Reporting and Output	International Seminar Fees	IDR 3,000,000
22	Reporting and Output	International Scientific Publication Fees	IDR 3,500,000
Total			IDR 155,607,000

B. Clinical Trials

Research methods	
1.	A summary of the research proposal includes the reason/motivation for conducting the research, the aims/objectives and benefits of the research, as well as the risks that may arise along with how to overcome them (written in language that is easy for non-doctors to understand): Reason/Motivation for Conducting Research: One nutritional strategy for obese individuals to lose weight is diet modification. Previous studies have shown that moderate weight loss (5–10% of initial body weight) achieved through lifestyle changes is associated with improvements in the cardiometabolic abnormalities characteristic of obesity (Espeland et al. 2007; Zomer et al. 2016). There have been studies and meta-

analyses that examine FTO gene polymorphisms coupled with certain types of dietary intervention which are then associated with changes in nutritional status, body composition, and various inflammatory and hormonal biomarkers in obese subjects (Razquin et al. 2010; De Luis et al. 2012b; De Luis et al. 2015a; De Luis et al. 2015b; Luglio et al. 2017; Di Renzo et al. 2018; De Luis et al. 2020; Parastouei et al. al. 2020), however, some things are still unclear and limited. Apart from that, no one has ever linked the FTO gene polymorphism to the Balanced Nutrition Diet intervention which is the reference in Indonesia, as well as its effect on cardiovascular risk.

Research purposes:

General purpose:

Examining the Effect of a Balanced Nutritional Diet on Cardiovascular Risk Factors Among Two Types of FTO rs9939609 Gene in Obese Young Women.

Special purpose:

1. Analyze differences in the body composition of obese young women between A allele carriers (AC) and TT allele homozygous (TH) groups after Balanced Nutrition Diet intervention
2. Analyze differences in biomarkers of inflammation related to cardiovascular risk (interleukin (IL-6), *high-sensitivity CRP* (hs-CRP), and adiponectin (ADP)) of obese young women between A allele carriers (AC) and TT allele homozygous (TH) groups after Balanced Nutrition Diet intervention

Benefits of research:

For research subjects, subjects can find out the allele type of the FTO rs9939609 gene themselves as an illustration of genes related to obesity and that can be used as a basis for precision nutrition interventions and will receive nutritional education about balanced nutrition. For the public, it is hoped that this research can increase knowledge about consumption recommendations, especially to normalize nutritional status and reduce the risk of cardiovascular disease by improving health status, especially for young adult women. For educational institutions, it is hoped that this research will provide new scientific evidence in the field of nutrigenomics regarding the interaction of genes and diet influencing the risk of obesity and cardiovascular disease. For the government, it is hoped that it can become a reference for making a policy to reduce obesity cases in Indonesia.

Number of Research Subjects:

This research uses a hypothesis testing formula for 2 independent samples based on Sastroasmoro & Ismael (2014):

$$n = n_2 = 2\left[\frac{(Z_\alpha + Z_b)S}{X_1 - X_2}\right]^2$$

The results of calculations using data from previous research (De Luis et al. 2015b) resulted in 7 people. This study used 4 groups, namely 2 control groups and 2 intervention groups, adding an anticipated dropout of 50%, the total number of subjects was at least 44 people or 11 people per group.

Information:

n = minimum number of subjects

Zα = type I error (1.96)

Zb = type II error (1.28; power 90)

S = standard deviation of the two groups

X1 – X2 = desired clinical difference (difference in weight between Allele T and Allele A)

Research by De Luis et al. (2015b) is a consideration for the expected weight loss of subjects in this study, namely assuming a weight loss of ±1 kg in A allele carriers and ±0.35 kg in homozygous T subjects after 1 month of intervention with the balanced nutritional diet provided.

How to Choose Research Subjects

<table>
<tr><td></td><td>Inclusion Criteria: 1) Obese women (body fat percentage >35%) who have light physical activity based on PAL (can select prospective subjects who have participated in preliminary research to completion); 2) Sundanese ethnicity (father, mother, grandfather, grandmother originally from West Java); 3) normal fasting blood pressure and blood sugar; 4) aged 18-25 years (based on Loos and Yeo (2014) that the relationship between the FTO gene and obesity is greatest in young adulthood); 5) not currently pregnant or breastfeeding; 6) willing to participate and sign informed consent.
Exclusion Criteria: 1) currently pregnant and breastfeeding; 2) have a history of chronic disease; 3) regularly consume antioxidant supplements and/or phytopharmaceuticals; 4) currently participating in other research.

Risks that may arise and how to overcome them:
Potential harm to the procedure being performed is very rare. The potential that may be encountered is limited to conditions of infection and the formation of hematomas (blood clots due to needle sticks). This risk can be reduced by treatment by experienced professionals and the use of disposable equipment and aseptic procedures, as well as appropriately sized needles to minimize the occurrence of hematomas. Apart from that, there may be a danger of bacterial contamination from intervention foods made by researchers. This can be prevented by maintaining hygiene and sanitation during preparation, processing, packaging, and when providing intervention food. In addition, the possibility of side effects or dangers from consuming the dietary intervention provided is prevented by collecting data on dietary restrictions/allergies for each subject before the intervention is carried out.</td></tr>
<tr><td>2.</td><td>Research Methods (There may be more than one) √ Randomization

√ Open labelled ☐Placebo ☐Cross-over
☐Single Blind √ Treatment controlled √ Parallel
☐Double Blinds ☐Others mentioned,</td></tr>
<tr><td>3.</td><td>New Drug Research (Investigational New Group=IND)/New Equipment (Investigational New Equipment =INE)/New methods or techniques:
<table>
<tr><td>√ There isn't any</td><td>☐There are:</td><td>☐IND
Reg No. POM:
Name:
Sponsor: Company name:</td><td>☐INE
Reg No. POM:
Name:
Sponsor: Company name:</td><td>Health Department Permit:
☐There is
☐No

If there is, attach it</td></tr>
</table>
Information About Try Ingredients:
a. Efficacy: –
b. Security: –</td></tr>
<tr><td>4.</td><td>For Genetic Research, Indicate Whether Genetic Engineering Techniques Are Used:
<table>
<tr><td>√ No:</td><td>☐Yes, it has been reviewed ☐Recombinant DNA Advisory Committee
☐Biosafety Committee</td></tr>
</table></td></tr>
<tr><td>5.</td><td>
<table>
<tr><td colspan="2">This data is filled in if it concerns clinical drug trials</td><td>Drugs Tested</td><td>Companion Medicine</td></tr>
<tr><td>a.</td><td>Generic name:</td><td></td><td></td></tr>
<tr><td>b.</td><td>Trade name:</td><td></td><td></td></tr>
<tr><td>c.</td><td>Chemical name:</td><td></td><td></td></tr>
</table></td></tr>
</table>

	d.	Pharmacology class:		
	e.	Dosage form and drug strength:		
	f.	Packaging:		
	g.	Way of giving:		
	h.	Expired date:		
	i.	Batch Number:		
	j.	Analysis certificate:		
	k.	CPOB Certificate:		
	l.	Type and quantity of medicines to be imported:		
	m.	Manufacturer's name and address:		
	n.	Importer name and address:		
	o.	Distribution status of the test drug in other countries (if any, please attach it)		
	p.	Clinical trial phase: ☐Phase I ☐Phase II ☐Phase III ☐Phase IV		

6.	How to Get Research Subjects:	☐Individual contacts √ Basic data from other studies ☐Reference☐Advertisement (attach)☐Others, explain

7.	Subject Criteria:	
	☐Prone to	☐Child's age < 12 years ☐Prisoner ☐People with impaired cognitive function/unconsciousness ☐Pregnant mother ☐TNI and Police ☐Others, please specify: ....
	√ Not Vulnerable	

8. If this research is a clinical trial using human subjects, have pre-clinical trials/tests on animals been carried out, data on safety and efficacy from previous studies/in other countries)? √ No☐Yes
References (Name, Title, Journal): –
Resume of Research Results: –

Previous experience (own or others) of the action to be carried out (have clinical trials on humans been carried out, safety and efficacy data from previous studies/in other countries): √ No☐Yes
References (Name, Title, Journal): Di Renzo L, Cioccoloni G, Falco S, Abenavoli L, Moia A, Sinibaldi Salimei P, De Lorenzo A. 2018. Influence of FTO rs9939609 and Mediterranean diet on body composition and weight loss: A randomised clinical trial NCT01890070 NCT. J Transl Med. 16(1):1–12. doi:10.1186/s12967-018-1680-7.
Resume of Research Results: Randomized clinical trial examining the effects of FTO rs9939609 and the Mediterranean diet on body composition and weight loss. The results obtained show that the Mediterranean Diet is proven to be good for reducing body fat mass, while the data on the effects of FTO are still uncertain.

9. Clinical Trial Process

a) Intervention administration (dose regimen, invasive and non-invasive procedures, comparison drugs, placebo):
Following informed consent and initial screening, blood samples will be collected from all research participants to establish baseline data. Subsequently, randomization of the samples will

be conducted using the block randomization method, based on the FTO gene allele. This process will involve two sample randomization blocks: one for A carriers allele and another for T homozygous individuals. Each block will then be randomized and divided into two groups, maintaining a 1:1 ratio between control and dietary intervention. Following randomization, four groups will be formed: two groups receiving the Balanced Nutrition Diet intervention (DAC: A allele carriers + Diet; and DTH: homozygous T allele + Diet), and two control groups (CAC: A allele carriers; and CTH: homozygous T allele). Nutrition education will be provided to all groups. The intervention period will span 4 weeks (28 days), after which a second blood draw will be conducted to gather endline data.

The Balanced Nutrition Diet is meticulously designed to supply the body with nutrients in suitable types and quantities, adhering to the principles of food diversity which include variety, balanced proportions, correct amounts, and consistency. Additionally, it emphasizes the "Fill My Plate" guideline, advocating for the consumption of all five food groups daily or at each meal. Portion sizes of the Balanced Nutrition Diet are structured according to the "Balanced Nutrition Tumpeng" model, encompassing 3-4 portions/day of carbohydrates, 2-3 servings/day of fruit, 3-4 servings/day of vegetables, and 2-4 servings/day of side dishes, with restrictions set at 4 tablespoons of sugar, 1 teaspoon of salt, and 5 tablespoons of oil per day, alongside a recommendation of drinking 8 glasses of water daily.

During the intervention period, food is provided three times a day - breakfast, lunch, and dinner - following a 28-day menu cycle. The researcher bears the sole responsibility for covering the cost of all food provided to the intervention group during this 28-day period. The Balanced Nutrition Diet offered adheres to specific macronutrient distribution ratios of 45-55% carbohydrates, 15-20% protein, and 20-25% fat, as outlined by Almatsier (2010). Complex carbohydrates, such as whole grains, are prioritized, while protein sources lean towards low-fat options, with half derived from plant-based sources. Monounsaturated and polyunsaturated fats are favored, and organic ingredients are preferred whenever possible.

Each meal's composition adheres to the "Fill My Plate" principle, and daily portions follow the "Balanced Nutrition Tumpeng" concept. Specifically, the diet provided by Eat & Fit Catering adheres to certain guidelines, including cooking methods that eschew fire and oil frying in favor of boiling, sautéing, or roasting techniques. Canola oil is the preferred oil, sodium-reduced salt is utilized, and mushroom stock serves as the primary flavouring agent, ensuring both healthfulness and taste. For each subject, energy requirements were calculated according to resting energy expenditure (REE) estimates using Mifflin-St. Jeor Equation (Mifflin et al. 1990). REE is then multiplied by the physical activity level (PAL) according to WHO-FAO that has been obtained.

REE (women)= (9.99 x Body Weight) + (6.25 x Height) + (5 x Age) – 161

The dietary distribution to subjects occurs three times, facilitated by couriers from the catering party. Should the delivery fall beyond the catering courier's radius, online delivery services will be utilized, with costs borne by the researcher.

In contrast, the control group subjects do not adhere to a specific diet regimen. However, they will receive general guidance on healthy eating habits and will be monitored closely. Nonetheless, their energy requirements will still be assessed based on REE and physical activity levels, as previously mentioned.

b) Determination of outcome indicators:

Evidence shows that compared with TT wild type, the risk allele A FTO rs9939609 showed significantly higher body weight, body mass index, waist circumference, hip circumference, and waist-to-hip ratio (Mangge et al. 2011; Tupikowska-Marzec et al. al. 2019; Mehrdad et al. According to Chrostowska et al. (2013), evidence is growing that the waist-hip circumference ratio is a stronger indicator of cardiovascular disease than body mass index. The meta-analysis of Gholamalizadeh et al. (2020), showed that carriers of the A allele of the FTO rs9939609 polymorphism had a higher fat percentage. The research results of Dorling et al. (2021) showed

the FTO SNP rs9939609 was associated with changes in REE adjusted for fat mass and lean mass, with the AA allele showing a decrease compared to TT.

Several studies have been conducted looking at the relationship between the FTO rs9939609 genotype and interleukin-6 (IL-6) levels, but only a few have shown real differences (de Luis et al. 2012b; Zimmermann et al. 2011; de Luis et al. 2016; Magno et al. al. 2018; Hosseini et al. Studies have also been conducted that there is an association of FTO rs9939609 with higher levels of C reactive protein (CRP) (Fisher et al. 2009; Sun et al. 2010; Lappalainen et al. 2011; Tupikowska-Marzec et al. 2019). However, other studies show contradictions (Zimmermann et al. 2011).

Research by Mehrdad et al. (2020a) showed that subjects with A allele carriers (AA and AT) had lower adiponectin levels than wild-type subjects (homozygous TT) with homozygous AA allele types having the lowest values although not significantly different. Several other studies also show the same thing in both obese and other types of adult subjects (Saucedo et al. 2017; Isgin-Atici et al. 2021). However, research by de Luis et al. (2016) and Duicu et al. (2016) showed a contrast, namely that adiponectin levels were lower in the TT genotype group compared to the AA genotype group. Based on what has been explained above, several outcome indicators that will be analyzed are waist-hip circumference, mass and total fat percentage &*visceral*, interleukin-6 levels, hs-CRP levels, and adiponectin levels.

Statistical analysis

The data obtained were tested statistically using SPSS statistical software for Windows (IBM version 26.0, SPSS Inc., Chicago, III., USA). The normality of variable distribution was verified with the Shapiro-Wilk test. Analysis of Variance (ANOVA) was used for all baseline data. Paired samples t-test was used to see changes in calorie intake, macronutrients, BW, BMI, waist circumference, hip circumference, waist-hip ratio, RM, percent total fat, visceral fat, subcutaneous fat, skeletal muscle, IL-6, hs-CRP, and adiponectin between before and after intervention. Data are presented as mean ± SD and $P < 0.05$ was considered statistically significant.

c) Planned interim analysis: There is no interim analysis in this study.

d) Clinical trial termination procedures: Referring to the guidelines for good clinical trials in Indonesia (POM RI 2016), clinical trials are terminated if clinical trial safety problems occur, such as serious undesirable events and serious side effects of the test product. The principal investigator will report the KTDS to the ethics committee no later than 3 days after the incident is discovered. The possibility of KTDS that could occur in this study is that the patient requires hospital treatment as a result of the intervention process.

e) Estimated research time required for one subject per procedure: ±4 weeks

f) Ethical issues (state your opinion about the ethical issues this research may encounter):

- *Respect for person* (respect for human dignity):
  Subjects in this study took part based on their own conscious choice, there was no element of coercion.
  Subjects have the right to be protected, including that all Subject data will be kept confidential.
- *Beneficence*(beneficial) Non-maleficence (not harmful):
  This research optimizes benefits and minimizes harm to subjects.
  The results of the research will be useful for society.
  The research design has been made as clear as possible.
  Researchers have competencies that are appropriate to the research topic to be conducted.
- *Justice*(justice):
  Subjects are treated the same/or there is no differentiation between subjects.

	There is no conflict of interest
10.	*Adverse Event reporting plan*(AE) a. Recording (What will be reported): Each incident must be recorded in detail regarding the time it occurred and the causes to avoid similar incidents and as additional data for research progress reports. b. Analysis and action procedure: 1. Anthropometric Measurements After an overnight fast for 12 hours, all subjects underwent anthropometric evaluation. All individuals were instructed to remove their shoes and wear as light clothing as possible before undergoing measurements. Body weight, mass & percent body fat were measured with a Bioelectrical Impedance Analyzer (BIA). Body height was evaluated using a stadiometer to the nearest 0.1 cm. BMI is calculated using the formula = body weight/height2 (kg/m2). Waist circumference and hip circumference were assessed using a flexible steel metric tape to the nearest 0.5 cm. The waist-to-hip ratio (WHR) was analyzed and evaluated according to clinical risk thresholds, equivalent to WHR > 0.9 for men and WHR > 0.85 for women. 2. Blood Sample Analysis A venous blood sample of around 6-10 cc is taken using a sterile syringe by competent licensed medical personnel. The blood is then divided into two different sterile tubes (Vacutainer®). The first tube is a purple cap tube containing EDTA, while the second tube is a yellow cap tube containing separator gel. After that, the sample is put on ice to be immediately taken to the testing site. The samples in the purple tube will then be processed for DNA and RNA isolation. DNA isolates were used for analysis of FTO gene variants using PCR and sequencing. Blood samples in yellow tubes were used for analysis of serum levels of interleukin-6, hs-CRP, and adiponectin using the ELISA method. All blood analyzes will be carried out at the Molecular Genetics Laboratory, FK UNPAD. The remaining samples were stored at −80 °C to be retested if necessary. c. *Emergency request system*: Each subject will be given a contact for researchers and doctors who can be contacted if there are complaints of illness related to the intervention. Researchers and doctors can be contacted 24 hours during the research period regarding interventions for emergency response preparedness. If there is an emergency, the subject will be taken to the emergency room at Hasan Sadikin Hospital. d. Termination of subjects in research due to adverse events: If an undesirable event occurs, it must be immediately reported to the researcher. Researchers will then coordinate with parties who feel it is necessary to carry out follow-up as soon as possible. Then the researcher will assess whether the subject needs to be discontinued in the study after consulting with a clinical trial expert.
11.	If this research uses human subjects, are the costs of dealing with side effects the responsibility of this research?☐No √ Yes - If yes, post-research responsibilities (capacity building, benefits to local community, continuation of therapy on the subject, etc.) - Is the subject insured? √ No☐Yes If "yes" please state the insurance institution: – Duration of Insurance and/or responsibility of the researcher towards the subject? –
12.	If we use biological samples, will they be sent overseas? √ No ☐Yes, please state the destination country and attach itdrafts*Material Transfer Agreement* (MTA)!

C. Process of Obtaining Approval After Explanation (INFORMED CONSENT)/Informed Consent (IC)

1.	To Whom INFORMED CONSENT Is Explained(There may be more than one answer):		☐Individual	√ Group	☐Guardian	
2.	a.	Who explains?		Principal Researcher		
	b.	When will it be explained?		Before INFORMED CONSENT screening and signing is carried out		
	c.	Are subjects given enough time to make a decision?		Yes		
	d.	Who signed the INFORMED CONSENT?		The prospective subject itself		
	e.	Who witnessed the INFORMED CONSENT signing?		Research assistant (doctor/nurse/midwife/nutritionist)		
3.	Ethical problems that the subject may face					
	a.	Research risks				
		1. Disrupts routine health service activities		☐Yes	√ No	
		2. Causes side effects on the subject		☐Yes	√ No	
		3. Contrary to norms and customs local		☐Yes	√ No	
		4. Economic losses and stigmatization occur subject		☐Yes	√ No	
	b.	Benefits of taking part				
		1. New knowledge increases		√ Yes	☐No	
		2. Get health services		√ Yes	☐No	
		3. Incentives		☐Cash☐No √ Goods/Souvenirs		
		4. Compensation		☐Insurance √ Cash ☐No☐Etc		
	c.	Excessive influence (coercion)				
		1. Relationship between Chief Researcher and subject		√ No	☐There is	
		2. If there is:	☐Doctor-patient	☐Teacher/lecturer-pupils/students	☐Superiors and subordinates	☐Etc
	d.	• If the study uses healthy people, explain how the health checks will be carried out: – • If the study uses sick people, explain how to diagnose and name the doctor responsible! This study used young adult female subjects who were obese. The diagnosis of obesity is assessed by a nutritionist based on the percentage of total body fat > 35%. Responsible doctor: dr. Lucky Angkawijaya Roring, M.Pd., AIFO. (08112228197).				

D. Fill in the Consent Information After Explanation (INFORMED CONSENT)/Informed Consent (IC)

1.	Is the Narrative in Agreement After the Subject Explanation Explaining About:			
	a. Research summary information	√ Yes	☐No	
	b. The treatment applied to the subject	√ Yes	☐No	☐Not Available
	c. Benefits for the subject	√ Yes	☐No	☐Not Available
	d. Potential danger	√ Yes	☐No	☐Not Available
	e. Right to resign	√ Yes	☐No	☐Not Available
	f. Incentives for subjects	√ Yes	☐No	☐Not Available
	g. Type of incentives provided	√ Yes	☐No	☐Not Available
	h. Compensation for subjects	√ Yes	☐No	☐Not Available

2.	Specimen Collection (Statements 2b-2d are filled in if the answer to 2a is "yes" and 2f-2g if the answer to 2e is "yes")		
	a. Was a specimen taken from the subject?	☐No	√ Yes, Blood
	b. Is there information on the number of specimens taken?	√ Yes	☐No
	c. Is there any information about the frequency of collection?	√ Yes	☐No
	d. Is there any information about how to collect it?	√ Yes	☐No
	e. Was there any invasive procedure on the subject?	√ No	☐Yes, Name it
	f. Is there any information about the potential risks of taking?	√ Yes	☐No
	g. Is there information about how to handle risk-taking?	√ Yes	☐No
3.	Subject Confidentiality		
	a. Is there any information about subject confidentiality?	√ Yes	☐No
	b. Is there any information about specimen confidentiality?	√ Yes	☐No
	c. Is there any information about data confidentiality?	√ Yes	☐No
4.	*Contact person local* for respondents, please specify: Putri Novitasari (08112227291)		
	Contact person centre for respondents, please specify: Putri Novitasari (08112227291)		

E. Statement

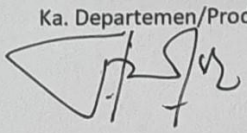
1.	Has the head of research been involved/punished for criminal/disciplinary action by the public or a private medical organization/an authorized body? √ No☐Yes, explain	
2.	How long will research data be kept by the Principal Researcher? 1 to 2 years after the research is completed	
3.	What precautions are taken to maintain the confidentiality of health data?	
	√	Research documents/files will be stored in a secure location and can only be accessed by officers involved in the research
	√	Computer data is only intended for officers involved in research and can be accessed using passwords and personal access
	√	Before accessing any research-related information, personnel must sign a consent form to protect the security and confidentiality of the subject's health information
	√	Before opening research files, officers must sign an agreement to maintain the confidentiality of the documents
	√	Where possible, identification of research subjects is removed (anonymous) from information related to research
	☐	Others, explain
4.	I will be responsible for filling out this form and will carry it out in accordance with the proposed research proposal and in accordance with the principles of research ethics.	

KOMISI ETIK PENELITIAN
RESEARCH ETHICS COMMITTEE
Jl. Prof. Eijkman No. 38 Bandung 40161
Telp. & Fax. 022-2038697, website: kep.unpad.ac.id, email-sekretariat: kep@unpad.ac.id

Bogor, 05 Juni 2023

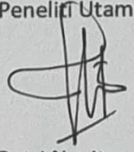
Mengetahui,

Ka. Departemen/Prodi



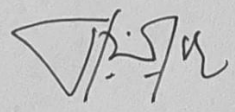
Dr. Drs. Rimbawan

Peneliti Utama,



Putri Novitasari, S.Gz., M.Si

Pembimbing I,



Dr. Drs. Rimbawan

Pembimbing II,

Prof. Dr. Ir. Hardinsyah, M.S.

Pembimbing III

Prof. Dr. Ir. Hadi Riyadi, M.S.

INFORMATION SHEET

The Effect of a Balanced Nutritional Diet on Cardiovascular Risk Factors Among Two Types of FTO Gene rs9939609 ini Obese Young Women

I am Putri Novitasari, a Doctoral (S3) student in Nutrition Science from the IPB University who is conducting research for her dissertation. I invite you to participate in this research, your participation in this research is voluntary, so you can decide whether to participate or not.

Research purposes:
The general aim of this research is to
Examining the Effect of a Balanced Nutritional Diet on Cardiovascular Risk Factors Among Two Types of FTO Gene rs9939609 in Obese Young Women.

Meanwhile, the specific aim of this research is to
1. Analyze differences in the body composition of obese young women between A allele carriers (AC) and TT allele homozygous (TH) groups after Balanced Nutrition Diet intervention
2. Analyze differences in biomarkers of inflammation related to cardiovascular risk (interleukin (IL-6), *high-sensitivity CRP* (hs-CRP), and adiponectin (ADP)) of obese young women between A allele carriers (AC) and TT allele homozygous (TH) groups after Balanced Nutrition Diet intervention

Why Subject was chosen:

You were selected for this study because you meet all the inclusion criteria, namely: 1) Have a fat percentage of >35% and have light physical activity based on PAL (and have participated in preliminary research to completion); 2) Sundanese ethnicity; 3) normal fasting blood pressure and blood sugar; 4) aged 18-25 years (based on Loos and Yeo (2014) that the relationship between the FTO gene and obesity is greatest in young adulthood); 5) not currently pregnant or breastfeeding; 6) willing to participate and sign informed consent.

Procedures/Procedures:

You will be invited to the UPT Health Services, Indonesian Education University gathered with other respondents at one time. First of all, an explanation of the aims and objectives of the research is provided, followed by an explanation of the actions/treatment that will be carried out in this research including the benefits and disadvantages of being a research subject including the compensation you will receive and the right to withdraw from the research. Then there will be a distribution of informed consent and if the prospective subject is willing and signs the informed consent and passes the screening, they will officially become research subjects.

The first stage of screening is assessment, physical examination (blood pressure, pulse and fasting blood sugar), and anthropometry (body weight, height, waist circumference and hip circumference, total & abdominal fat mass and percentage) as basic measurements for internal screening All prospective subjects had fasted overnight. If you do not pass the screening, then you will be declared disqualified. On the other hand, if you pass the screening then you are declared a research subject. Medical assessments including blood pressure and blood sugar checks by nurses/midwives and health checks by doctors are evaluated before and after the intervention.

Screening of potential subjects, signing of the INFORMED CONSENT, collection of subject blood specimens, and data collection were carried out at the UPT Health Services (Poliklinik), Indonesian Education University. Address: Jl. Dr. Setiabudhi No. 229, Isola, Sukasari, Bandung City

After that, on the same day, you will have a blood sample taken as initial biochemical data. After the initial biochemical data was obtained, samples were randomized using the block randomization method based on the FTO gene allele. There will be 2 sample randomization blocks, namely the A carriers allele block, and the T homozygous block. Each block was randomized and divided into 2 groups with a ratio of control to dietary intervention (1:1). Because this research uses a parallel design, you only experience one type of intervention. Randomization was carried out using a roll of paper containing subject codes without replacement. Randomization was carried out by personnel who did not participate in research activities. After that, they enter the intervention stage for 4 weeks (28 days) according to the group. After 4 weeks (28 days), a second blood draw will be taken as final biochemical data. Analysis of the FTO gene and other blood biochemistry will be carried out at the Molecular Genetics Laboratory, Faculty of Medicine, Padjadjaran University.

Height measurement uses a microtome with an accuracy of 0.1 cm, BB measurement uses a step scale with an accuracy of 0.1 kg. Waist circumference and hip circumference were measured using a body circumference meter with an accuracy of 0.1 cm. Mass and percentage of total & visceral fat using the Bioelectrical Impedance Analyzer (BIA) with an accuracy of ± 400 grams for a body weight of 0.0–40.0 kg, while for a body weight of 40.0–135.0 kg, the accuracy is ± 1%. Blood biochemical data include high sensitivity-C-reactive protein (hs-CRP); interleukin-6 (IL-6), and plasma adiponectin. Gene data examined includes FTO gene variations. Consumption data includes: recording food consumption (self-administered food record). Food records 2x24 hours were carried out before the intervention started and during the intervention. Subjects will be instructed to record or record the type and weight of food and drinks consumed and then the nutrients will be reviewed and calculated. Compliance with the consumption of a Balanced Nutrition Diet using Googleform which must be filled in by the intervention group every day. The minimum limit for subject compliance is 80% of the entire diet provided. Physical activity data includes the length of time doing various types of physical activity within 24 hours, used to estimate the subject's energy expenditure.

Food was provided during the intervention period 3 (three) times each day, namely breakfast, lunch and dinner. The food menu uses a 28-day menu cycle. The cost of all food provided to the intervention group during the 28 days was the sole responsibility of the researcher.

The characteristics of the Balanced Nutrition Diet that will be provided have a distribution of the composition of the macronutrients that will be intervened in are as follows: 45-55% carbohydrates, 15-20% protein, and 20-25% fat (Ministry of Health 2014). The type of diet and portions that will be given are the same for each feeding unless there are certain food restrictions or allergies, the type of food will be adjusted specifically to that individual. The calories that will be given range from 300-400 Cal for breakfast, 400-500 Cal for lunch, and 300-500 Cal for dinner.

The choice of carbohydrates should be varied and should be complex carbohydrates such as whole grains. The choice of protein type is prioritized from low-fat protein sources and half of it is vegetable protein. The choice of type of fat is prioritized in the form of monounsaturated and polyunsaturated fats. If possible, food ingredients should be prioritized in the form of organic food. The composition of each meal refers to the principle of "Fill My Plate" and the daily portion refers to the principle of "Balanced Nutrition Tumpeng". The characteristics of the diet that will be provided specifically from the Eat & Fit Catering diet consist of 1) Cooking methods that use boiling/sautéing/roasting techniques without fire and avoiding frying techniques

with oil; 2) The oil used is canola oil; 3) The salt used is a special type of salt which has less sodium content; 4) The flavouring used is mushroom stock.

For each subject, energy requirements were calculated according to resting energy expenditure (REE) estimates using Mifflin-St. Jeor Equation (Mifflin et al. 1990). REE is then multiplied by the physical activity level (PAL) according to WHO-FAO that has been obtained.

REE (women)= (9.99 x Body Weight) + (6.25 x Height) + (5 x Age) – 161

The technical distribution of diets to subjects is delivered 3 times by couriers from the catering party. If the delivery is outside the delivery radius of the catering courier, the food will be delivered by online delivery services and costs will be charged to the researcher.

In contrast, in the control group, subjects do not follow a specific diet, but they will also receive general recommendations about healthy eating habits and will only be monitored, however, their energy needs will still be evaluated based on REE and physical activity as mentioned above.

Risks and inconveniences:

The risks in this study are very small/minimal, but there is inconvenience because your time will be taken up for collecting data and recording dietary compliance (for those in the intervention group). Apart from that, the potential that may be encountered is limited to conditions of infection and the formation of hematomas (blood clots due to needle sticks). This risk can be reduced by treatment by experienced professionals and the use of disposable equipment and aseptic procedures, as well as appropriately sized needles to minimize the occurrence of hematomas. Apart from that, there may be a danger of bacterial contamination from intervention foods made by researchers. This can be prevented by maintaining hygiene and sanitation during preparation, processing, packaging, and when providing intervention food. In addition, the possibility of side effects or dangers from consuming the dietary intervention provided is prevented by collecting data on dietary restrictions/allergies for each subject before the intervention is carried out.

Benefits (direct to the subject and general):

For you, you can find out the allele type of the FTO rs9939609 gene as an illustration of genes related to obesity and which can be used as a basis for precision nutritional interventions and will receive nutritional education about balanced nutrition. For the public, it is hoped that this research can increase knowledge about consumption recommendations, especially to normalize nutritional status and reduce the risk of cardiovascular disease by improving health status, especially for young adult women.

Alternative procedure:

"There isn't any"

Data confidentiality:

Researchers will guarantee the confidentiality of research data.

Estimated number of subjects to be included:

The total number of research subjects who will take part in this research is around 44 people.

Volunteerism:

Subject participation is voluntary without any coercion and is accompanied by responsibility until the completion of the research.

Subject Participation Period:

You will be involved in the entire series of this research for approximately 2 months, consisting of 1 month intervention period and an additional 2 weeks each before and after the intervention period for preparation.

Subjects can be excluded/withdrawn from research:

Drop-Out Criteria are 1) you state that you do not wish to continue; 2) indications of exclusion criteria were found in you during the research; 3) You did not undergo complete blood and/or other necessary tests.

Possible funding from health insurance companies or researchers:
"no insurance was provided in this study"

Incentives and compensation:
Transport costs when you go to the screening site and sign the INFORMED CONSENT will be reimbursed in cash amounting to IDR. 50,000,-. Those who have participated until the final data collection will be given incentives in the form of contact materials in the form of boxes and cutlery.

Question:
If anything is unclear or you need to ask questions, please contact the principal investigator:

Putri Novitasari (08112227291) : D Amerta Residence Jl. Pandanus III Block E1 No. 30 Bojongsoang District, Bandung Regency

Contact a doctor if any side effects occur: Dr. Lucky Angkawijaya Roring, M.Pd., AIFO

APPROVAL AFTER EXPLANATION (INFORMED CONSENT)
TO PARTICIPATE IN RESEARCH
(INFORMED CONSENT)

I have read or received an explanation, am fully aware, understand and comprehend the objectives, benefits and risks that may arise in the research, and have been allowed to ask questions and have been answered satisfactorily, and can withdraw from participation at any time. then I agree/disagree*) take part in this research, entitled:

The Effect of a Balanced Nutritional Diet on Cardiovascular Risk Factors Among Two Types of FTO Gene rs9939609 in Obese Young Women

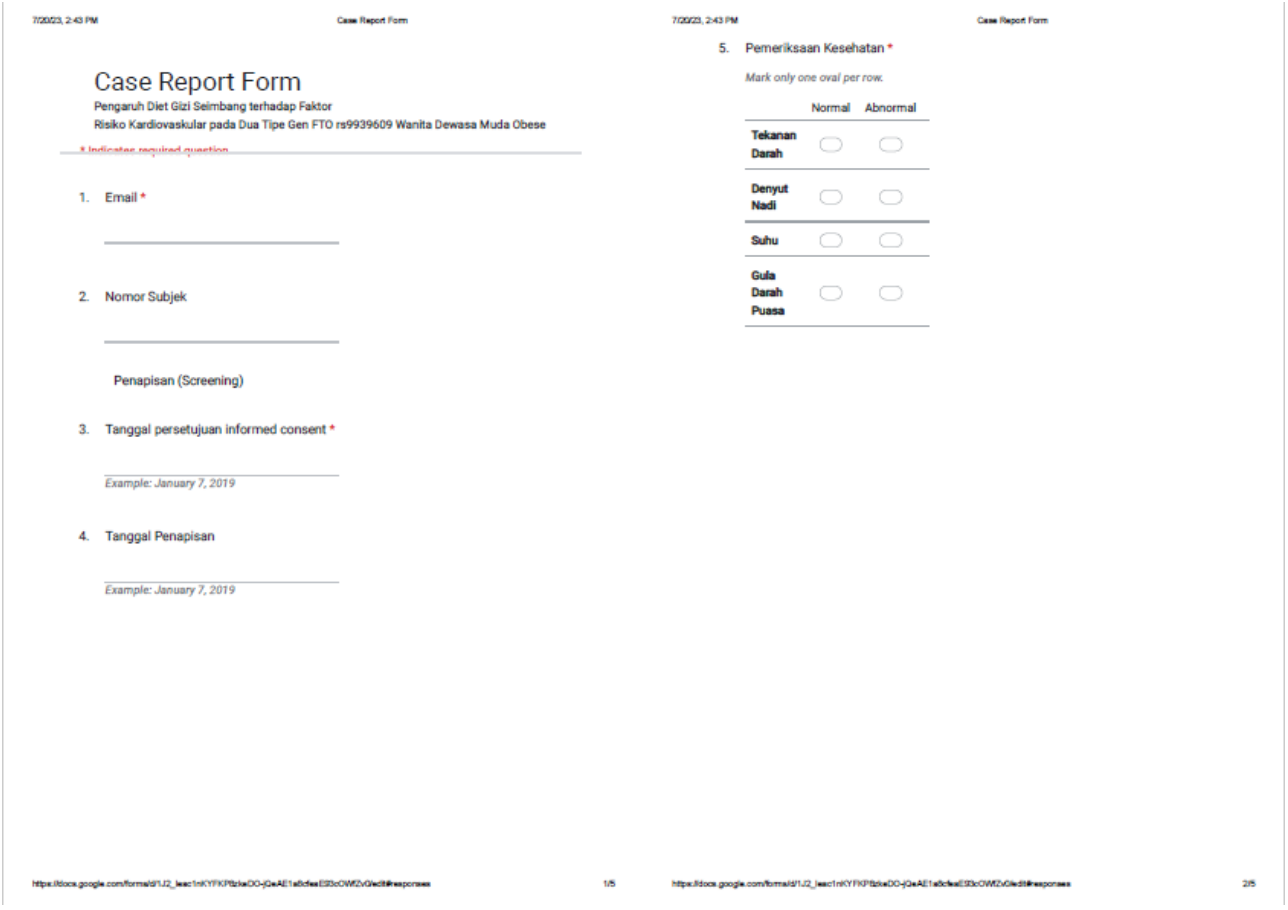
I voluntarily chose to take part in this research without pressure/coercion from anyone. I will be given a copy of the explanation sheet and the signed consent form for my records.
I agree:
Yes No[*)]

	Date:	Signature (if not available, thumbprint can be used)
Participant Name: Age: Address:		
Researcher Name:		
Witness Name:		

*) cross the unnecessary ones

CASE REPORT FORM

Case Report Form is available at:https://forms.gle/zsCVMFcFvAzZJ6HcA



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Case Report Form

Pengaruh Diet Gizi Seimbang terhadap Faktor Risiko Kardiovaskular pada Dua Tipe Gen FTO rs9939609 Wanita Dewasa Muda Obese

* Indicates required question

1. Email *

2. Nomor Subjek

Penapisan (Screening)

3. Tanggal persetujuan informed consent *

Example: January 7, 2019

4. Tanggal Penapisan

Example: January 7, 2019

https://docs.google.com/forms/d/1J2_leac1nKYFKPBzkeDO-jQeAE1a8cfeaES3cOWfZvGl/edit#responses 1/5

7/20/23, 2:43 PM Case Report Form

5. Pemeriksaan Kesehatan *

Mark only one oval per row.

	Normal	Abnormal
Tekanan Darah		
Denyut Nadi		
Suhu		
Gula Darah Puasa		

https://docs.google.com/forms/d/1J2_leac1nKYFKPBzkeDO-jQeAE1a8cfeaES3cOWfZvGl/edit#responses 2/5

8. Eksklusi *

Check all that apply.

	Ya	Tidak
Sedang hamil dan menyusui?	☐	☐
Memiliki riwayat penyakit kronis?	☐	☐
Rutin konsumsi suplemen atau obat-obatan fitofarmaka?	☐	☐
Sedang berpartisipasi pada penelitian lain?	☐	☐

6. Antropometri *

Mark only one oval per row.

	Nilai
BB aktual	⬭
TB aktual	⬭
IMT	⬭
Total persen lemak tubuh	⬭
Persen lemak visceral	⬭
Total massa lemak tubuh	⬭
Massa lemak visceral	⬭
Lingkar pinggang	⬭
Lingkar pinggul	⬭
Rasio lingkar pinggang-pinggul	⬭

Kriteria

7. Inklusi *

Check all that apply.

	Ya	Tidak
Obese (IMT>=30 kg/m2 dan persen lemak >35%)?	☐	☐
Etnis Sunda?	☐	☐
Usia 18-25 thn?	☐	☐
Tekanan darah dan gula darah puasa normal?	☐	☐
Bersedia dan menandatangani informed consent?	☐	☐

Research Ethics Committee
Universitas Padjadjaran

Certificate of Attendance

NO. B03/UN6.KEP/DL/2019

This is to certify that

Prof. Dr. Ir. Hardinsyah, MS.

has attended and completed

TRAINING ON GOOD CLINICAL PRACTICES

held in Bandung, 12 - 14 November 2019

Dhamayanti, dr., Sp.A(K)., M.Kes.
n of the Research Ethics Committee
Universitas Padjadjaran

Prof. Rovina Ruslami, dr., Sp.PD., Ph.D.
Course Coordinator
Universitas Padjadjaran

No: GCP-C/CRSUFMUI/06/2021/031

CERTIFICATE OF COMPETENCE

CRSU
CLINICAL RESEARCH SUPPORTING UNIT

This certificate is awarded to

Dr. Ir. Hadi Riyadi, MS

who has passed the competency test of

Good Clinical Practice

Organized by
Clinical Research Supporting Unit, Faculty of Medicine, Universitas Indonesia

Jakarta, 10 June 2021

Prof. dr. Franciscus D. Suyatna, PhD, SpFK
Head of CRSU FMUI