ADDIS ABABA UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

CENTER FOR FOOD SCIENCE AND NUTRITION

Assessment of the Effects of Periodic Fasting on Inflammation and Food Reward

By: Dawit Alelign

Advisor: Kaleab Baye (PhD)

December, 2018

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A Thesis Submitted to School of Graduate Studies, Addis Ababa University, in Partial Fulfillment of the Requirements for the Attainment of the Degree of Masters of Science in Food Science and Nutrition

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Lists of Abbreviations and Acronyms

AAU	Addis Ababa University
AD	Alzheimer Disease
ADF	Alternate-Day Fasting
AHRI	Armauer Hansen Research Institute
BMI	Body Mass Index
BP	Blood Pressure
CR	Caloric Restriction
CRP	C-Reactive Protein
CVDs	Cardiovascular Diseases
DM	Diabetes Mellitus
DR	Dietary Restriction
EPHI	Ethiopian Public Health Institute
GBD	Global Burden of Disease
HDL	High-Density Lipoprotein
HFSA	High Fat Savory
HFSW	High Fat Sweet
HR	Heart Rate
hs-CRP	High Sensitivity C-Reactive Protein
ICAM	Intercellular Adhesion Molecule-1
IL-6	Interleukin 6
LFPQ	The Leeds Food Preference Questionnaire
LFSA	Low Fat Savory
LFSW	Low Fat Sweet
LPS	Lipopolysaccharide
OFC	Orbit Frontal Cortex
PD	Parkinson's disease
SPSS	Statistical Package for Social Sciences
TFEQ-R18	The Three Factor Eating Questionnaire Revised 18-Item
TNF	Tumor Necrosis Factor
VCAM	Vascular Cell Adhesion Molecule-1
VLDL	Very Low Density Lipoprotein
WC	Waist Circumference
WHR	Waist to Hip Ratio

Abstract

Background: Increased urbanization and economic development have led to a shift to consumption of higher caloric diet. This dietary pattern is associated with markers of inflammation. Fasting plays a remarkable part in regulation of food intake. However, there is no study conducted on the role of fasting practice in relation to inflammation and food reward in Ethiopia.

Objective: To assess the effects of periodic fasting on inflammation and food reward.

Methodology: Before and after study design having controls was used among voluntarily selected participants and followed over Easter fasting period in two groups. The study populations for examining the effect of fasting on inflammation were fasters (n=42) and non-fasters or controls (n=38) groups. In addition, another fasters group (n=46) was involved in the study of effect of fasting on food reward. Participants were measured for serum high sensitive C reactive protein (hs-CRP) and anthropometric measurements before the beginning and at the end of fasting .Explicit liking, explicit wanting and 24 dietary recalls were collected at pre fast state and after the completion of fasting .The Three Factor Eating Questionnaire Revised 18 –Item (TFEQ-R18) traits were assessed only at pre fasting state. Paired t-test was used for comparison of baseline and end line measurements in each group. Besides, independent sample t-test was used for comparison of differences between groups. Bivariate correlations were used to evaluate associations between food rewards with TFEQ-R18traits.

Results: Between the baseline and end line assessments, although the changes were statistically insignificant, among fasters hs-CRP decreased by 22.9% .In addition, fasters involving in liking and wanting have showed an insignificant increase in high fat savory (HFSA) liking by 10.2%, low fat savory (LFSA) liking by 8.8%, high fat sweet (HFSW) liking by 24.4%, HFSA wanting by 5.2%, LFSA wanting by 8.3%, HFSW wanting by 9.3%. The 24 hour dietary recall results showed that the participants consumed more energy from HFSA and LFSA foods at post fasting than pre fasting even though the change was still statistically not significant. Furthermore, it was seen that uncontrolled eating was positively associated with explicit liking for high fat savory foods ($p<0.05$). Besides, emotional eating was positively and significantly associated with explicit liking and explicit wanting for HFSA, HFSW and LFSA foods.

Conclusion: Inflammatory marker (hs-CRP) was decreased and the food reward values were increased for most food categories (HFSA, LFSA and HFSW) among fasters after the end of fasting even though those presented differences were not statistically significant. This highlights that fasting might have a substantial positive impact on health if after fast cognitive controlling of eating is achieved.

Key words: Periodic fasting, explicit liking and wanting, Food reward, Inflammation, TFEQ-R18traits, 24 hour dietary recall

1. INTRODUCTION

1.1 Background of the Study

Non-communicable diseases like cardiovascular diseases (CVDs) are the leading causes of morbidity and mortality worldwide, causing nearly one-third of all deaths (1). According to the Global Burden of Disease (GBD) report for 2015, the prevalence of diabetes rose from approximately 333 million persons in 2005 to approximately 435 million persons in 2015, an increase of 30.6% (2) .Many sub-Saharan African countries are changing lifestyles which are related to the prevalence of CVDs risk factors including hypertension and obesity (3).Studies indicate that abdominal obesity is associated with higher levels of inflammatory markers (IM) including C-reactive protein (CRP), Interleukin-6 (IL-6), homocystein (Hcy), etc (4,5).

In the past, overweight and obesity were considered a problem of high-income countries; however, now days it is dramatically on the rise in low- and middle-income countries, particularly in urban settings (6). In Addis Ababa, the prevalence of overweight/obesity increased significantly by 28%; while underweight decreased by 21% between 2000 and 2011. Specifically, the prevalence of urban obesity increased by 43.3% i.e., from 3.0% to 4.3% in about 15 years (7).

Increased urbanization, westernization, and economic development have led to consumption of higher caloric diet and experiencing behavioral changes that are risk to health (3).Fasting plays a significant part in regulation of food intake that is characterized by a variety in the degree of caloric restriction and abstinence from specific foods (8). Although relevant data are still limited, previous studies have pointed towards a positive effect of fasting on the prevention of cardiovascular disease, type 2 diabetes mellitus and obesity (9).

A serum level of inflammatory markers (CRP, IL-6, and TNF-) predicts the onset of cardiovascular events, and individuals with high levels of all those three markers had the greatest risk of cardiovascular events (10). These markers(cytokines) are significantly higher in obese individuals than non-obese individuals(11).On the other hand ,recent attention has started to focus on the hedonic determinants of eating behavior and has highlighted the importance of distinguishing liking (i.e., the perceived pleasurable sensory properties of food) from wanting

(i.e., the attraction towards a specific food over available alternatives) (12,13). Both components of food reward are thought to act in parallel to facilitate eating behavior (14). Heightened liking and wanting for food have been reported in overweight and obese individuals (15). The reinforcing value of food has demonstrated that 'wanting' and 'liking' can change in. hungry and satiated states (16). Although choosing to eat in ways that meet longer-term goals of promoting a healthy and desired body weight is prominent, food deprivation and restriction increase the desire of food consumption if conscious intake regulation is not implemented (18).

Therefore, the present study is aimed to assess the effects of periodic fasting on inflammation and food reward considering the practice of Ethiopian Orthodox Easter fasters as a case.

1.2 Statement of the Problem

Type 2 diabetes and CVD are major diseases that cause deaths worldwide (1, 2). Previous studies have pointed a positive effect of fasting on the prevention of cardiovascular disease, type 2 diabetes mellitus and obesity. However, the relevant data are still limited. Inflammatory markers like, CRP appears to be predictor of Type 2 diabetes and CVD (17).This study will clarify the effect of fasting on inflammation. To the best of the researcher's knowledge, there is no study conducted on the role of fasting practice in relation to inflammation in Ethiopia.

On the other hand, calorie restriction and/or deprivation may potentially contribute to over-eating, thereby reducing the effectiveness of obesity treatment. Acute food deprivation is associated with increased consummatory behavior both in animals and humans (18). Variation in food culture can affect fat and sweet preferences, which are influenced by genetic and environmental factors (19).Thus, more work remains to be performed to show clearly compensatory eating behavior following periodic fasting in Ethiopia.

1.3 Significance of the Study

Serum Inflammatory markers are risk factors for Type 2 diabetes, CVD and other diet related diseases. The effect of periodic fasting on the markers is not clearly known. Such knowledge will have important implications to improve the effectiveness of prevention of diseases. Besides, the findings of the study will show the extent to which people are attracted to eat high fat and sweet food after ending the prescribed fasting period. This will highlight the importance of successfully

resisting overconsumption (consuming higher energy density foods in larger proportion of meal) in order to achieve a better standard of health. Furthermore, the study will provide baseline data for elucidating the effect of periodic fasting on inflammation and food reward as it will serve as background information for further future studies.

1.4 Objectives

1.4.1 General Objective

- The objective of this study is to assess the effects of periodic fasting on inflammation and food reward.

1.4.2 Specific Objectives

- To measure the effect of fasting on hs-CRP
- To examine the effect of fasting on food reward (liking and wanting components)
- To assess eating behavior traits (cognitive restraint, uncontrolled eating and emotional eating)
- To describe the effect of fasting on dietary patterns

2. LITERATURE REVIEW

2.1 Fasting

Fasting is a partial or total abstention from foods. According to Trepanowski& Bloomer (8), the most commonly investigated fasts are :I) **Caloric restriction (**energy intake reduction by mostly20-40%), ii) **Alternate day fasting (**rotating "feast period" and "fast period), and iii) **Dietary restriction (**reduction of mostly macronutrient components of dietary intake with little or no decrease in total energy intake). The Great lent or *Abye Tsome* fasting is characterized by periodic abstinence of any animal or dairy source foods and skipping of meals (caloric restriction) (20).

Generally, it is indicated that fasting has a potential to improve health. For instance, different fasting types were found to play significant roles in prevention of cardiovascular disease, cancers, kidney disease, diabetes and neurodegenerative diseases (21-26).

2.2 Inflammatory Markers and their Role in Human Disease

A role of inflammation in atherosclerotic disease process is discussed over the past decade (27-28). The stages of inflammation (initiation, growth and complication) are all inflammatory responses to injury (29-30).The major factors that promote atherogenesis are cigarette smoking, hypertension, atherogenic lipoproteins, and hyperglycemia. These risk factors elicit secretion of both leukocyte soluble adhesion molecules, which facilitate the attachment of monocytes to endothelial cells, and chemo tactic factors. This encourages the monocytes' migration into the subintimal space (31).Monocytes may differentiate into macrophages. Macrophages may take up deposited atherogenic lipoproteins through scavenger receptors and be transformed into foam cells (32).

Macrophages, mast cells, and activated T cells accumulate within the growing atherosclerotic lesion. Oxidized low-density lipoproteins may contribute to loss of smooth muscle cells through apoptosis .The secretion of metalloproteinase and other connective tissue enzymes by activated macrophages may break down collagen, which in turn weakens the cap and rupture it. This exposes the atheronecrotic core to arterial blood, which induces thrombosis (31).

Thus, inflammation involves cytokines and other bioactive molecules. Potential targets for measurement of inflammatory process include oxidized low-density lipoproteins, proinflammatory cytokines (eg, interleukin-1, tumor necrosis factor-), adhesion molecules (eg, intercellular adhesion molecule-1, selectins), inflammatory stimuli with hepatic effects (eg, interleukin-6) or the products of the hepatic stimulation, such as C-reactive protein (CRP), and a host of other acute-phase reactants. Furthermore, elevated leukocyte count might be assessed. In addition to cardio vasculature, inflammatory cascade may occur at other body parts (eg, gingivitis, prostatitis, bronchitis, urinary tract infections, and gastric inflammation) (31).

Serum C-reactive protein (CRP), tumor necrosis factor (TNF)-a, and interleukin (IL)-6 have been implicated in atherogenesis .CRP has been shown to be an important marker of vascular inflammation and a predictor of atherosclerosis. Adipose tissue is an important source of cytokines. Excess adiposity increased serum cytokine levels in human and animals. In obese subjects, CRP was significantly correlated with BMI and IL-6 was significantly associated with visceral adiposity (11).

In general, CRP levels are observed to be increased during acute-phase inflammation as well as chronic inflammatory diseases. From both experimental and clinical data, increasing evidence suggest that elevated CRP concentrations are associated with an increased risk of cardiovascular diseases ,type 2 Diabetes Mellitus, Alzheimer's disease, hemorrhagic stroke, Parkinson's disease, and Age-related macular degeneration (33). Recent studies demonstrate the capability of elevated hs-CRP to predict coronary events in women. In acute coronary syndromes, hs-CRP predicts recurrent myocardial infarction and seems to predict prognosis and recurrent events in patients with stroke and peripheral arterial disease (11, 34). The following are some of the diseases and health problems related to inflammation:

Diabetes: Inflammation plays an essential role in the development of insulin resistance and type 2 diabetes mellitus.A high intake of rapidly digested and absorbed carbohydrates can induce rapid postprandial glucose and insulin responses, leading to an insulin-resistant state characterized by hyperinsulinemia and dyslipidemia (ie, high triacylglycerol and low HDL concentrations). C-reactive protein and interleukin-6 levels have been shown to predict type 2 diabetes in humans (35-38).

Alzheimer disease (AD): Higher spontaneous production of interleukin 1 or tumor necrosis factor α by peripheral blood mononuclear cells may be a marker of future risk of Alzheimer disease (AD) in older individuals. The elevated CRP level was associated with an increased risk of AD (44).These data strengthen the evidence for a pathophysiologic role of inflammation in the development of clinical AD (40).

Parkinson's disease (PD): Previous studies have highlighted the key role of neuroinflammatory reactions in the pathogenesis of PD and patients with PD were shown to exhibit higher levels of serum hs-CRP (33).

Hypertension: Hypertension follows closely behind lipids on a list of classical risk factors for atherosclerosis. Like atherosclerosis itself, inflammation may participate in hypertension providing a pathophysiological link between these two diseases. (41).

Obesity: Obesity predisposes to insulin resistance, diabetes and atherogenic dyslipidemia. High levels of free fatty acids originating from visceral fat reach the liver and stimulate synthesis of the triglyceride-rich lipoprotein VLDL by hepatocytes. The resulting elevation in VLDL can lower HDL cholesterol by exchanging from HDL to VLDL by cholesteryl ester transfer protein. Adipose tissue can also synthesize cytokines such as TNF- and IL-6. In this way obesity itself promotes inflammation(42).

Infection: Acute infections can alter hemodynamic, the clotting and fibrinolytic systems in ways that can precipitate ischemic events. Chronic extra vascular infections (eg, gingivitis, prostatitis, bronchitis, etc) can augment extra vascular production of inflammatory cytokines that may accelerate remote atherosclerotic lesions. (43).

2.3. Relationship between Food Intakes and Markers of Inflammation

Dietary patterns are associated with biochemical markers of inflammation and endothelial activation. The fats and processed meats pattern (fats, oils, processed meats, fried potatoes, salty snacks, and desserts) are positively associated with CRP and IL-6. In contrast, the whole grains and fruit pattern (whole grains, fruit, nuts, and green leafy vegetables) are inversely associated with CRP and IL-6 (44). Consumption of *trans* fatty acids, stearic acid and saturated fatty acids diets increased CRP, fibrinogen, and IL-6 concentrations (45).In normal subjects, the high-fat

meal increased the plasma levels of tumor necrosis factor- (TNF-), interleukin-6 (IL-6), intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), which were prevented by vitamins. In addition, a single high-fat meal in human induces endothelial dysfunction which, at least in coronary circulation, predicts adverse cardiovascular events and long-term outcome (46).On the other hand, high fat diet-fed mice are associated with the increased hepatic expression of proinflammatory cytokines, including TNF and IL-6 (47).

There is increased circulating plasma endotoxin after a high-fat meal in healthy subjects. Increased postprandial endotoxin or lipopolysaccharide (LPS) may contribute to the development of the postprandial inflammatory state, endothelial cell activation, and early events of atherosclerosis (48).

Therefore, it is recommended to modify food choices to reduce saturated fats (10% of calories), cholesterol (300 mg/d), and trans-fatty acids by substituting grains and unsaturated fatty acids from fish, vegetables, legumes, and nuts. It is also important to limit salt intake to 6 g/d and alcohol intake (2 drinks/d in men, 1 drink/d in women) among those who drink (49).

Increased consumption of fat, particularly saturated fat, has been linked to increased plasma concentrations of lipids, insulin resistance, glucose intolerance and obesity. Because low carbohydrate diets derive large proportions of calories from protein and fat, there has been considerable concern for their potentially detrimental impact on cardiovascular risk. A very low carbohydrate diet (carbohydrate 60 g d-1) is more effective than a low fat diet (30% or less daily energy from dietary fat) for resulting in profound alterations in fatty acid composition and reduced inflammation (50-53).

Relative to other carbohydrate sources, sugar intake appears to be associated with increased triglyceride levels, a known risk factor for coronary heart disease and a higher consumption of high-sugar beverages and foods is associated with evidence of increased inflammation and oxidative stress (54).

The consumption of foods and beverages containing fructose does not stimulate insulin secretion from pancreatic cells. Because leptin production is regulated by insulin responses to meals, fructose consumption also reduces circulating leptin concentrations. The combined effects of

lowered circulating leptin and insulin in individuals who consume diets that are high in dietary fructose could increase the likelihood of weight gain. In addition, fructose is more preferentially metabolized than glucose in the liver. Fructose consumption induces insulin resistance, impaired glucose tolerance, hyperinsulinemia, and hypertension in animal models. The data in humans are less clear (55).

2.4. Fasting and Food Reward Behaviors

Feeding behavior has been shown to be modulated by hunger state, whereby prolonging inter-meal intervals. Food reward contributes to the pleasure and motivation to obtain food (16).

The hedonic value of food (food reward) is described as a combination of how much a food is liked and how much a food is wanted in a given moment. These psychobiological constructs have a primary role in how much energy is consumed. Moreover the processes of liking and wanting for food are not always equivalent and may vary depending on the food in question, state of satiety, body composition and individual differences in dispositional eating behavior traits (56).

2.4.1. Food Reward Processing

It has been explained that both the amygdala and the orbit frontal cortex (OFC) play an important role in forming reward values (57). The amygdala and the OFC are densely interconnected and carry out their reward functions they interact with other dopaminergic mesocorticolimbic structures (the striatum, the dopaminergic midbrain and the anterior cingulate cortex) (58-59). Behavior might be affected by amygdala as it provides a memory link between a stimulus and its incentive value. This information is sent to the OFC to predict reward outcomes (60).

In addition, the ventral tegmental area and nucleus accumbens motivate the reward of food intake. The palatability of available food can undermine normal satiety signals, motivating energy intake independent of energy need. The food intake is increased when Sucrose flows directly into the nucleus accumbens and changes dopamine and opioid neurotransmission. Both sweet and high-fat foods mobilize opioids and dopamine within the nucleus accumbens and cause excess energy intake. Chronic amygdala activation by stress increases cortisol secretion and promotes palatable food consumption as a form of self-medication (54).

Calorie content of food and hunger are factors that influence reward processing in the lateral and medial OFC, cingulate cortex, caudate putamen, insula and fusiform gyrus (57).Reduced calorie foods suppressed ratings of hunger for several hours after consumption (61).

2.4.2. Food Deprivation and Restriction

Food deprivation is a reduction of overall caloric intake, and food restriction refers to conditions in which cognitive or environmental constraints have been implemented so as to limit access to certain foods (18).

History of food deprivation may influence current eating behaviors similar to acute food deprivation (62-64). For example, men who have decreased intake to around 1500 kcal/day for 24-weeks exhibited uncontrollable eating and increased appetites for sweet foods (65).Therefore, deprivation and restriction may affect consummatory behavior by increasing the desire to obtain food. It has also shown that food deprivation decreases self-control (66-67).

3. METHODOLOGY

3.1 Study Area and Period

The research was conducted in Addis Ababa; the capital city of Ethiopia, from January 25 to April 12, 2018.The study was specifically carried out in six sub cities-Gulelie, kolefie keraniyo, Addis ketema, Lideta, Bole and Yeka.

According to the Ethiopian National Population and Housing Census of 2007, Addis Ababa has a total population of 2,737,551. About 23% of the total urban population of Ethiopia lives in Addis Ababa and with respect to religion 74.7% are Orthodox Christians, 16.2% Islam and 7.8% are Protestants (68).

3.2 Study Design

Community based before and after study design having controls with selected participants from eligible population and followed over Easter fasting period in two groups; those who fast (exposed) and non-fast (control) group. The study assessed the effect of fasting on inflammation and food reward. For this, hs-CRP and anthropometric measurements were made for two groups before the beginning (pre or baseline) and at the end of the Easter fasting period. Explicit liking, explicit wanting and 24 dietary recalls were collected at pre fast state and after the completion of fasting .The Three Factor Eating Questionnaire Revised 18 –Item (TFEQ-R18) traits were assessed only at pre fasting state.

For the assessment of explicit liking, explicit wanting ,24 dietary recalls andTFEQ-R18traits, female subjects were excluded in order to feasibly collect the data by avoiding the influence that the luteal phase of the menstrual cycle may have on energy intake and food choice (69-70).

3.3 Study Population, Study Subjects and Sampling Procedure

Study populations were Orthodox Christians living in Addis Ababa. The study subjects were Orthodox Christians chosen using convenience sampling technique within the community that were willing to participate and satisfying the inclusion criteria of the study.

3.4 Eligibility Criteria

3.4.1 Inclusion Criteria

Volunteers, aged 18 years and older giving consent for the study were used. Subjects who were permanent residents or temporarily residing in Addis Ababa during the study period were included. The faster group consisted of the study participants that fasted during the Easter fasting period .For the control group, those subjects that did not fast during the great lent fasting were included. Those who were apparently health were included (71).To make food preferences free of bias, those subjects without food allergies were included.

3.4.2 Exclusion Criteria

Those who were with illnesses and taking long term medications were excluded. Those who were unwilling to participate in the study; lactating and pregnant women were excluded.

3.5 Sample Size Determination

G Power 3.0.10 software was used to calculate sample size (72-73).It requires selecting appropriate test family (t-test in our case), type of statistical test within test family (dependent sample t-test), specifying α error probability, power (1-β error probability), and determining effect size. Considering medium effect size (0.5); power (1-β) of the study (0.85) and α-error probability (0.05) the sample size were 38 for each group.

38 x 2 (Control and Fasting group) = 76 considering 20% non-response, 16 subjects were added and the total number of study subjects were 92 (46 fasters and 46 controls).

3.6 Data Collection and Instruments for Data Collection

Subjects were examined and measured 1 week before the beginning (pre) and the last 5 days before the completion (end) of the Easter fasting period with regard to assessment of inflammation. Evaluation of food reward was carried out 3 weeks before the fasting period and four days after end of fasting. This used a test meal consisted of 219gram (503 kcal)of donut and 500liter of bottled water at pre fasting ; and 72.8 gram (128.8 kcal) of cheese pizza at post

fasting . No particular criteria were applied to the selection of these meals since the manipulation was designed only to bring subjects to similar satiated state (74).

Questionnaires

All volunteers participating in the study were interviewed face-to-face .The questionnaire had four components: questions on socio-demographic characteristics and general fasting related questions; The Three Factor Eating Questionnaire Revised 18-Item (TFEQ-R18); The Leeds Food Preference Questionnaire (LFPQ); and 24 dietary recalls.(Annex 4).

TFEQ-R18 was used to assess cognitive restraint, uncontrolled eating and emotional eating as described by Karlsson *et al* (75) and De Lauzon*et al* (76) .The theoretical ranges for the items were 6-24 for cognitive restraint, 9-36 for uncontrolled eating and 3-12 for emotional eating (77).

LFPQ was used to assess liking and wanting for food using photographic images of common food items. This behavioral task had been widely used in previous researches (13, 14, 16, and 19) .In the current study, the TFEQ-R18and the LFPQ were translated to Amharic by a teacher of English as a foreign language and the translation was approved by nutrition experts. Twenty five LFPQ photographic stimuli were prepared. Of these images, sixteen best LFPQ photographic stimuli were chosen(See Annex 2).Stimuli were categorized according to fat content (high or low) and taste (sweet or savory). To measure explicit liking, participants were required to rate "how pleasant would it be to taste some of this food now? The ratings for each food item were averaged for each food type. To measure explicit wanting, Participants were required to respond to "How much do you want some of this food now?"The experimental examination of liking and wanting was configured by administering the stimuli or food items (measuring 258 X 193 mm2) on laptop computer. Participants made their ratings using a 100-mm line scale (16). The ruler that was used for measuring ratings of the subjects had $\pm$0.001 precision values.

By a 24-h food recall, participants were asked to provide detailed information regarding what they had eaten and how it was prepared, along with serving size information. Food items and amounts from the 24-h recalls were entered into Nutrisurvey 2007, and the caloric intake; grams of moisture, protein, fat, carbohydrate, zinc, calcium, iron, thiamine, riboflavin, niacin, vitaminA and vitamin C were obtained.

Anthropometric assessments

Body weight was measured two times at pre-stage and end of fasting by a digital scale to the nearest 100g, placed on flat surface. Subjects were weighed barefoot in very light clothing. Standing height was measured without shoes to the nearest 0.1 cm with the shoulders in relaxed position, arms hanging freely, feet together, heels against the back board and knees straight. Body Mass Index (BMI) was calculated by dividing weight (kg) by height squared (m2). Waist circumference was measured at the midpoint between the lower margin of the least palpable rib and the top of the hip or minimal waist using stretch-resistant tape. Hip circumference was measured around the widest portion of the buttocks, with the tape parallel to the floor. For both measurements, the subject stood with feet close together thereby body weight was evenly distributed, arms at the side and wearing light clothing. When the subject became at relaxed state, measurements were taken at the end of normal expiration (78).

Serum hs-CRP measurements

Subjects were told to fast overnight, blood samples were obtained in the morning after 12 hours over night fast from an antecubital vein. Blood samples of 6-10 ml were collected in to plain vacuum tube, using proper sanitation and infection prevention techniques and after 30 minutes, the collected blood samples were centrifuged at 3000 revolution per minute for 10 minutes; the separated serum were placed in cold box carrier containing ice packs that maintained the temperature around –40°C (79-80) .The serum were later used to determine participants' hs-CRP. The serum hs-CRP levels were measured using Roche/Hitachi cobas c 311, cobas c 501/502 (Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim Germany) at Ethiopian Public Health Institute, clinical chemistry laboratory.

3.7 Data Analysis

A statistical analysis program SPSS (version 20 for Windows) was used to analyze the data. Frequency distribution of socio-demographic characteristics of the study population was determined. Independent sample t test and Non-parametric Mann-Whitney test were used to evaluate the difference in the distribution of categorical variables of the study groups. The Shapiro-Wilk test was performed to assess the normality of the distribution. For normally

distributed variables, within group comparisons of pre and end fasting test results were analyzed using paired t-test for fasting or the control group separately. Whereas, independent samples t-test was used after obtaining differences in result values before and at the end for each group to compare with difference of difference test. Food hedonics was analyzed according to fat content of food images (high-fat or low-fat) and taste of food images (savory or sweet).

Bivariate correlations were used to evaluate associations between explicit liking and wanting components of food reward with TFEQ – R18 uncontrolled eating ,cognitive restraint and emotional eating .Continuous variables were presented as mean values $\pm$ standard deviation, while categorical variables were presented as absolute numbers and relative frequencies. All differences were considered statistically significant at $p < 0.05$.

3.8 Operational Definitions

Fasters are defined as those who have been fasting regularly and decided to fast the Easter fasting period through avoiding consumption of all animal source foods during the period (81).

Obesity is defined as a condition of abnormal or excessive fat accumulation in the adipose tissue of the body which is explained by BMI value > 30kg/m2 (80).

Cut-off points of high sensitivity C-reactive protein (hs-CRP)) for CVD risk assessment :<1 mg/L (low risk); 1.0-3.0 mg/L(average risk); > 3.0 mg/L (high risk) (34)

3.9. Study Variables

3.9.1. Dependent Variable

Anthropometric measurements (body mass index, waist circumference and waist to hip ratio); hs-CRP level, 24 dietary recall assessment, explicit liking, explicit wanting, eating behavior traits (cognitive restraint, uncontrolled eating and emotional eating)

3.9.2. Independent Variable

The independent variables were individual's position based on fasting status; either fasting or non-fasting. In addition, socio-demographic characteristics such as age, sex, educational and income status

3.10. Ethical Considerations

Ethical clearance was obtained from AAU, college of natural and computational sciences review board for ethical approval with Reference number CNSDO/255/10/2018; Dated January 25, 2018 (See Annex 1). At the time of data collection, a written consent was taken from the participants to confirm whether they were willing to participate or not. Those not willing to participate were given the right to do so. Information was collected after securing consent from study participants. Confidentiality of responses was also ensured throughout the research process. Data obtained from each study participant were kept confidential and all who participated in the study were acknowledged.

3.11. Dissemination of Results

The results of this study were submitted and presented to Addis Ababa university center of food science and nutrition as partial fulfillment of a master's degree in food science and nutrition. The findings will also be disseminated through publishing on national or international journal and presentations on scientific conferences.

4. RESULTS

4.1. Scio-demographic characteristics and fasting practice

As shown in Table 1, Socio- demographic characteristics of the study participants for examining the effect of fasting on inflammation were volunteers that constituted fasters and non-fasters (controls) groups, with mean age of 31.1±7.7 and 30± 5.8, respectively. There were no statistically significant differences between fasters and controls group with respect to age, educational status and monthly income, with p values 0.827, 0.164 and0.732, respectively. During the fasting period, all the fasters avoided animal source food; furthermore, 61.91% skipped breakfasts and28.57% fasted until 3pm.

Table 1: Socio-demographic characteristic of the study subjects, January 2018, Addis Ababa

Variables	**Fasters N (%)**	**Controls N (%)**	**p-value**
Sex			0.015
Males	19 (45.2%)	30 (78.9)	
Females	23 (54.8%)	8 (21.1)	
Age (Mean ± Stan Dev.)	31.1 ± 7.7	30±5.8	0.827
Educational status			0.164
Elementary school/No education	9 (21.4)	10 (26.3)	
Secondary school	11 (26.2)	18 (47.4)	
>Secondary school	22 (52.4)	10 (26.3)	
Monthly income			0.732
<900 Eth Birr	10 (23.8)	8(21)	
1000-1999 Eth Birr	9 (21.4)	8(21)	
2000-2999 Eth Birr	6 (14.3)	6 (16)	
>3000	17 (40.5)	16(42)	

P - Values are from comparison between fasters and controls using Non-parametric Mann-Whitney test.

In addition, there were forty-six participants involved in food reward, 24 dietary recalls and the TFEQ – R18 items assessments with mean age of 27.2±3.2.All the participants were males, having learnt in colleges and universities and earning a minimum of net 3000 Ethiopian birr per month. They all abstained from animal source food. Besides, 56.25% jumped breakfasts and 14.58% fasted up to 3 pm.

4.2. Baseline Characteristics of Study Participants

Baseline characteristics of hs-CRP and anthropometric measurements between fasters and controls group are presented in Table 2 and there were no statistically significant differences between the two comparison groups with respect to hs-CRP and all anthropometric measurements.

Table 2: Baseline characteristics of hs-CRP and anthropometric Measurements between fasters and controls groups, February 2018, Addis Ababa

Variables	Fasters		Controls		p-value
	Mean	St. Dev.	Mean	St. Dev.	
hs-CRP*(mg/L)	2.58	3.6	1.15	0.7	0.479
BMI*(kg/m^2)	22.5	3.9	22.15	4.9	0.498
Weight (kg)	60.6	12.5	63.1	13.4	0.473
WC* (cm)	83.3	11.6	81.9	11.2	0.668
WHR	0.85	0.1	0.85	0.1	0.818

hs-CRP, high sensitivity C-reactive protein; BMI, body mass index; WC, waist circumference; WHR, waist to hip ratio. *Non-parametric Mann-Whitney test was used.

4.3. Comparisons of Pre and End Line-fasting Values in the Fasting and Control Groups

The results of hs-CRP and anthropometric measurements between baseline and end line among fasting and control groups are shown in Table 3. In the fasting group, although the changes were not statistically significant, there were declines in the level of serum hs-CRP by 22.9%, weight by1.8 %, BMI by1.8%, waist circumference by 2.3 %, and waist to hip ratio by 1.2 %.

In control group, there were statistically insignificant increase in the level of hs-CRP by 8.7%, waist circumference by 1.1% and waist to hip ratio by 1.2%.But, weight and BMI were decreased by 0.2%even though the differences were still statistically not significant.

Table 3: Hs-CRP and anthropometric measurements between baseline and end line fasting & control groups, April 2018, Addis Ababa

Variables	Fasters			Controls		
	Baseline	End line	p-value	Baseline	End line	p-value
hs-CRP *	2.58±3.6	1.99±2	0.833	1.15±0.7	1.25±1.9	0.258
BMI *	22.5±3.9	22.1±3.8	0.552	22.15±4.9	22.10±5.1	0.908
WEIGHT	60.6±12.5	59.5±12.2	0.682	63.1±13.4	63.0±13.8	0.971
WC*	83.3±11.6	81.4±11.3	0.383	81.9±11.2	82.8±11.0	0.686
WHR	0.85±0.1	0.84±0.1	0.394	0.85±0.1	0.86±0.1	0.702

Values are presented as mean ± standard deviation, hs-CRP: high sensitivity C- reactive protein; BMI: body mass index, WC: Waist circumference, WHR: waist to hip ratio, *Non-parametric Mann-Whitney test was used.

4.4. Comparisons of Changes in the Fasters and Controls Groups

Changes between groups are presented in Table 4 showing statistically significant differences in BMI (p = 0.033), weight (p = 0.035), WC (p < 0.001) and WHR (p <0.001); however, the value for hs-CRP was shown insignificant between group difference.

Table 4: Mean difference comparison of hs-CRP and anthropometric measurements between fasting and control groups, April 2018, Addis Ababa

Variables	Fasters		Controls		p-value
	Mean diff	SEMD	Mean Diff	SEMD	
hs-CRP (mg/L)	-0.59	0.46	0.1	0.40	0.67
BMI(kg/m^2)	-0.4	0.08	-0.05	0.14	0.033
Weight (kg)	-1.1	0.22	-0.1	0.39	0.035
WC (cm)	-1.9	0.30	0.9	0.38	<0.001
WHR	-0.01	0.00	0.01	0.00	<0.001

P-values are from comparison between fasters and controls using non-parametric Mann-Whitney test, SEMD: standard error of the mean difference, hs-CRP: high sensitivity C-reactive protein, BMI: body mass index, WC: waist circumference, WHR: waist to hip ratio.

4.5. Explicit Liking

As illustrated in figure1, although the effects were not statistically significant, there were increases in the LFPQ explicit liking scores from the pre fast to post fast ：HFSA by 10.2%, LFSA by 8.8%, and HFSW by24.4%.However, there was insignificant decline in explicit liking score for LFSW by4.3%.

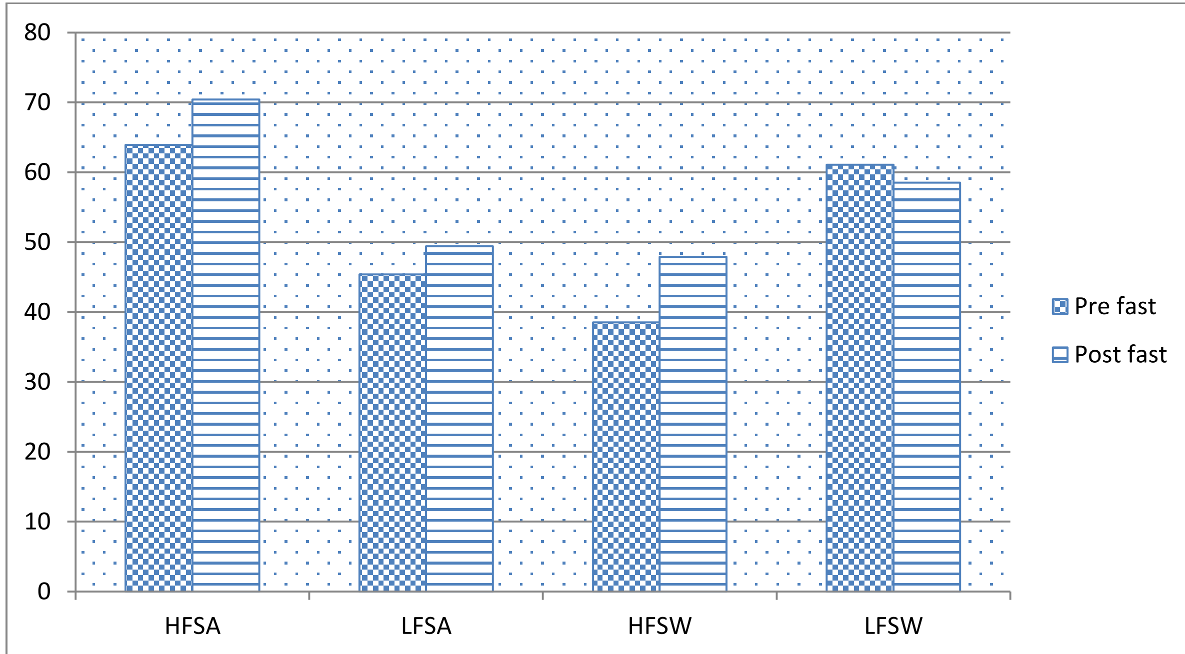

Figure 1: Results from the Leeds Food Preference Questionnaire for liking of food images at pre fasting and post fasting conditions, April 2018, Addis Ababa. Showing mean scores for each of the four food categories: high fat savory (HFSA), low fat savory (LFSA), high fat sweet (HFSW) and low fat sweet (LFSW).

4.6. Explicit Wanting

Even though the differences were not still significant, there were increases in the LFPQ explicit, wanting scores from pre fast to post fast: HFSA by5.2%, LFSA by8.3%,and HFSW by9.3%. But, there was insignificant decrease in explicit wanting score for LFSW by 5.9%. (Seefigure2)

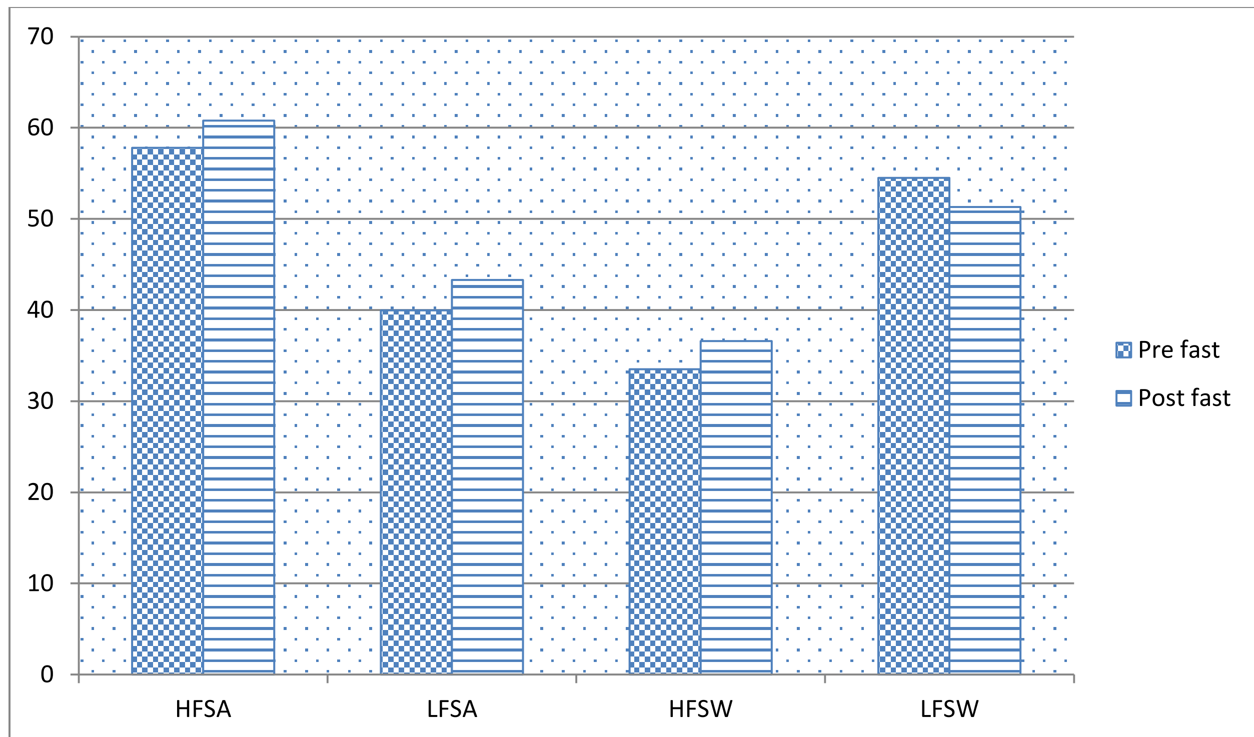

Figure 2: Results from the Leeds Food Preference Questionnaire for wanting of food images at pre fasting and post fasting conditions, April 2018, Addis Ababa. Showing mean scores for each of the four food categories: high fat savory (HFSA), low fat savory (LFSA), high fat sweet (HFSW) and low fat sweet (LFSW)

4.7. Associations with TFEQ Eating Behavior Traits

Mean scores± standard deviation (maximum and minimum in parentheses) for the Three Factor Eating Questionnaire Revised 18 items (TFEQ-R18) scores were 10 ± 2.98 (6-24), 17.8 ± 4.6 (9-36), and 3.9 ± 1.4 (3-12), for cognitive restraint, uncontrolled eating, and emotional eating, respectively.

As can be seen in Table 5, bivariate correlations revealed that: explicit liking for HFSA was positively associated with TFEQ – R18 uncontrolled eating under pre fasting condition (r=0.347, P< 0.05), and explicit liking for LFSA and HFSW were positively correlated with emotional eating (r=0.335, P< 0.05; r=0.388, P< 0.01, respectively), under pre fasting condition. In addition, explicit liking for HFSA was positively associated with TFEQ-R18 emotional eating under post fasting condition (p<0.01).Explicit wanting for LFSA was positively correlated with TFEQ-R18 uncontrolled eating at post fasting (r=0.299, p<0.05); and explicit wanting for HFSA and HFSW were positively associated with emotional eating (r=0.298, P<0.05; r= 0.352, P<

0.05, respectively) at pre fasting state. In addition, explicit wanting for LFSA was positively associated with TFEQ – R18 emotional eating under post fasting condition (r=0.289, p< 0.05). There were no other significant corrections between variables.

Table 5: Associations between explicit liking and explicit wanting for the four categories at pre fasting and post fasting conditions with uncontrolled eating, cognitive restraint and emotional eating, April 2018, Addis Ababa

Condition	Category	Uncontrolled Eating		Restraint		Emotional Eating	
		Explicit Liking	Explicit Wanting	Explicit Liking	Explicit Wanting	Explicit Liking	Explicit Wanting
Before Fasting	HFSA	0.347*	0.202	-0.005	-0.013	0.181	0.298*
	LFSA	0.014	0.086	-0.057	-0.062	0.335*	0.377
	HFSW	-0.112	-0.003	0.139	0.164	0.388**	0.352*
	LFSW	0.118	0.093	0.201	-0.122	0.186	0.118
After Fasting	HFSA	0.105	-0.019	0.236	0.118	0.409**	0.208
	LFSA	0.271	0.299*	-0.099	-0.100	0.245	0.289*
	HFSW	-0.025	-0.038	0.241	0.244	0.204	0.165
	LFSW	0.064	-0.005	-0.192	-0.058	-0.004	-0.056

*P< 0.05, **P<0.01, high fat savory (HFSA), low fat savory (LFSA), high fat sweet (HFSW) and low fat sweet (LFSW)

4.8. Twenty Four Hour Recall Energy and Nutrient Intake

The 24 hour dietary recall results are shown in Table 6, the participants consumed more energy from HFSA food at post fasting [924.5 (480,1278)] than pre fasting [892 (335.5, 1417.5)] although the change was not significant. And, they consumed a greater number of calories from LFSA food at post fasting [1724 (1426.75, 2091.50)] than pre fasting [1676 (1283, 2110)].

Table 6: Energy and nutrient intake of 24 hour dietary recall at pre fasting and post fasting, April 2018, Addis Ababa

Energy & Nutrients /day	Food category	Dietary intake [Median (Q1, Q3)]		P-value
		Pre fasting	Post fasting	
Energy (kcal)	HFSA	892 (335.5, 1417.5)	924.5 (480, 1278)	0.683
	LFSA	1676 (1283, 2110)	1724 (1426.75, 2091.50)	0.65
	HFSW	353.5(314, -)	310.5 (70.75, 596.75)	1
	LFSW	105 (52, 175)	105 (52, 236)	0.307
Water (g)	HFSA	304 (130.5, 462.5)	307 (103.5, 475)	0.715
	LFSA	1819 (1248, 2196)	1950.50 (1545.25, 2442)	0.118
	HFSW	29.5 (26, -)	26 (0, 88.75)	1
	LFSW	0 (0,73.5)	0 (0, 269.5)	0.613
Protein (g)	HFSA	54 (24, 149.5)	78.5 (35, 144.5)	0.212
	LFSA	48 (32, 59)	44.5 (36.25, 57.75)	0.752
	HFSW	5.5 (5, -)	5.5 (0.25, 12.25)	1
	LFSW	0 (0,0)	0 (0, 1)	0.181
Fat (g)	HFSA	36 (22, 63.5)	38.5 (22, 55.75)	0.802
	LFSA	20 (11,33)	17.5 (13, 23.75)	0.589
	HFSW	12.5 (11,-)	8 (4, 18.75)	0.533
	LFSW	0 (0,0)	0 (0, 0)	0.158
Carbohydrate (g)	HFSA	25 (12, 43.5)	21.5 (8.25, 44)	0.452
	LFSA	317 (246, 396)	333.5 (272.5, 404)	0.520
	HFSW	56 (50, -)	54 (9.25, 98)	1
	LFSW	27 (14, 43)	27 (14, 61)	0.350
Vitamin A(μg)	HFSA	170(9, 192)	97.5 (3.25, 288.25)	0.816
	LFSA	53 (48, 145)	53 (8, 146.5)	0.964
	HFSW	0 (0, 0)	0 (0, 0)	1
	LFSW	0 (0, 0)	0 (0, 0)	0.689
Vitamin B_1(mg)	HFSA	0 (0, 0)	0 (0, 1)	0.7
	LFSA	2 (1, 2)	2 (1, 2)	0.926
	HFSW	0 (0, 0)	0 (0, 0)	1
	LFSW	0 (0, 0)	0 (0, 0)	1

Energy & Nutrients/day	Food category	Pre fasting	Post fasting	P-value
Vitamin B2 (mg)	HFSA	1 (0, 1)	1 (0, 1)	0.386
	LFSA	1 (1, 1)	1 (1, 1)	0.877
	HFSW	0 (0, 0)	0 (0, 0)	1
	LFSW	0 (0, 0)	0 (0, 0)	0.3
Vitamin C (mg)	HFSA	10 (5, 21.5)	5.5 (3, 13)	0.045
	LFSA	14 (8, 21)	15 (10.25, 33)	0.094
	HFSW	0 (0, 0)	0 (0, 0)	1
	LFSW	0 (0, 0)	0 (0, 1.5)	0.612
Calcium (mg)	HFSA	151 (87.5, 325.5)	174 (83.75, 384.25)	0.725
	LFSA	744 (517, 906)	697 (502.75, 992.25)	0.982
	HFSW	101 (90, -)	25 (5, 145.5)	0.533
	LFSW	0 (0, 6)	0 (0, 19)	0.598
Iron (mg)	HFSA	17 (6, 53)	27 (9, 58.75)	0.264
	LFSA	84 (52, 103)	74.5 (53.25, 97.75)	0.589
	HFSW	2 (2, 2)	2.5 (0.25, 6.25)	1
	LFSW	0 (0, 0)	0 (0, 1.5)	0.088
Zinc (mg)	HFSA	10 (4, 27)	12 (6, 20)	0.358
	LFSA	10 (7, 13)	12 (8.25, 14)	0.101
	HFSW	0.5 (0, -)	0.5 (0, 1)	1
	LFSW	0 (0, 0)	0 (0, 0)	0.275

P - Values are from comparison between pre fasting and post fasting using Non-parametric Mann-Whitney test

5. DISCUSSION

The present study examined the effects of fasting on inflammation and food reward. Accordingly, hs-CRP and anthropometric characteristics decreased among fasters than controls although the changes were not statistically significant .Between the baseline and end line assessments, among fasters hs-CRP decreased by 22.9% .This finding of decreased value is in line with the results reported by previous studies(82,83,84,86).Harvie *et al* (82) who compared Intermittent Energy Restriction(IER) and Continuous Energy Restriction (CER) in 54 young overweight women found that high sensitivity C reactive protein reduced after 6 months of intervention. Imayama *et al* (83), found a significant reduction in serum level of hs-CRP (36.1%,$p<0.001$) among dieting overweight and obese post-menopausal women compared with controls .Likewise, Heilbronn *et al* (84) reported a significantly decreased CRP (5.56± 0.36 to4.12± 0.36 by 26%) after 12 weeks of energy restriction and weight loss on very low fat diets in obese healthy women(48± 0.9 years, MI 33.8±0.4kg/m2).In comparison to Heilbronn et al (84), the baseline concentration of CRP is lower in the present study. This is probably explained by high level of obesity in Heilbronn *et al* (84) study subjects which is in turn associated to increasing in CRP.

The increased production of CRP in obesity is most likely related to increased production of interleukin 6(IL6).Excess adiposity in obese people causes large production of IL6 (85).In turn, IL6 activates larger production of CRP from the liver.

Similarly, Lettieri *et al* (86) found that CRP and IL6 displayed a significant reduction after dietary restriction (DR).Bastard *et al* (87) found that IL6 but not CRP was reduced after a very low calorie diet had been imposed for 3 weeks. Here, CRP level showed reduction though it was not significant, similar to the results of the present study .The difference in the results of these studies and other studies with significant changes could be related to smaller number of subjects .Li *et al* (88) investigated on metabolic and psychological responses to 7 days fasting in 30 female obese patients (49± 8.1years, BMI30.4± 6.7 kg/m2).They found that hs-CRP slightly but significantly increasing (0.2± 0.2 to 0.5± 0.3, $p<0.001$).This disparity in findings may have arisen from mild stress or hermetic reactions of the shorter period of fasting .This is related with

increased circulating levels of catecholamine and thereby possibly leading to an acute phase response (89).

The anthropometric measurements decreased during the fasting period among fasters group ,but the changes were not statistically significant .Body weight and BMI were reduced by 1.8%.There were reduction of 2.3% in waist circumference and 1.2% in WHR .These decline trends are supported by the findings of previous studies(82,88,90). Harvie *et al* (82) in continuous energy reduction(N=54) found that weight reduced from 84.4 to 83.4 (in 1 month) ; 84.4 to 81.4 (in 3 months) ; and significantly from 84.4 to 79.9 (in 6 months) and they found that waist circumference reduced from 102.5 to 101.3 (in 1 month) ; 102.5 to 99.8 (in 3 months) ; and significantly 102.5 to 98.6 (in 6 months).Hadded *et al* (90), studying vegans and non-vegans have found a significantly lower BMI levels in the vegetarians group. Li *et al* (88) reported significantly decreased weight and BMI after 7 days of fasting.

In the current study, the participants' ratings of explicit liking and explicating wanting for LFPQ food categories were greater in post fasting compared to pre fasting state , except for decreased scores to LFSW foods .Between the baseline and the post fasting ratings , among fasters HFSA liking increased by 10.2%,LFSA liking by 8.8% ,HFSW liking by 24.4%, HFSA wanting by 5.2% ,LFSA wanting by 8.3%,HFSW wanting by 9.3% .In contrast to this , LFSW liking declined by 4.3% and LFSW wanting by 5.9%. However, all these findings were not statistically significant. The increased values of the current study are in line with previous works of LFPQ measurements for short fasting periods and fed states (15, 20).Though it is not statistically significant, the highest change (HFSW liking by 24.4%) is in agreement with imaging studies showing a robust activation of brain reward areas to sucrose taste (91) and to images of high calorie foods (92), after a period of fasting. The reason for the changes not to be statistically significant is probably due to manual ratings instead of using computerized procedure or the delayed post fasting measurements .The end line food reward assessment was made after four days from the completion of fasting period just for controlling the Ester holiday effect. In the current study, the decreased explicit liking and wanting for LFSW foods after fasting may be attributable to sensory specific satiety to the pizza test meal participants consumed. After the savory pizza, sweet foods could be tended to be preferred (93, 94).

When the correlation between the LFPQ scores and the TFEQ trait results were examined, it was seen that uncontrolled eating is positively associated with explicit liking for high fat savory foods. This finding is in line with previous report that has shown those who score high on disinhibition (uncontrolled eating) tend to exhibit a greater liking for high fat foods (95).Besides, emotional eating was positively associated with explicit wanting for HFSA and HFSW at pre fasting; and for LFSA under post fasting condition.

6. STRENGTH AND LIMITATIONS OF THE STUDY

Levels of inflammatory biomarkers have been implicated to predict adverse cardiovascular events and other non-communicable diseases which are presently growing very rapidly in low-income countries being mediated by diet-induced overweight/obesity. Therefore, the current study is timely as it helps to figure out the effect of periodic fasting on those inflammatory markers and food reward. In addition, the study used within subject design that minimizes inter individual variability and potentially helps to see differences before and after fasting. Furthermore, a parallel control group was also used to consider any environmental variations.

The study had also some limitations that should be considered. Firstly, the findings from the 24 hour dietary recall procedure may not be representative of habitual eating behavior as only one measure was taken. The procedure would be more representative if conducted on at least three different occasions. Secondly, the participants' food intake was assessed using dietary recall rather than being objectively measured under, laboratory conditions, which may have an impact on validity of these results. Thirdly, a computer assisted assessment was not used while conducting food reward measurement, which might in turn affect the data quality. Finally, the food reward assessment did include homogenous participants without employing respective controls.

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7. CONCLUSIONS AND RECOMMENDATIONS

Inflammatory marker (hs-CRP) decreased after periodic fasting although the change was not significant. For better understanding, further studies are required to examine the effect of fasting on more powerful inflammatory markers (IL6 and TNF) in larger study populations. In addition; the effects of multiple seasons fasting need to be studied.

Relative to pre fasting state, the food reward measures were increased for most food categories (HFSA, LFSA and HFSW) after the fasting period. The 24 hour dietary recall results showed that the participants consumed more energy from HFSA and LFSA foods at post fasting than pre fasting. Unlike the findings seen in most related studies, these changes were statistically insignificant. Therefore, future studies should examine the food preferences in more controlled manner.

Furthermore, it was seen that uncontrolled eating was positively and significantly associated with explicit liking for high fat savory foods. Emotional eating was positively and significantly associated with explicit liking and explicit wanting for HFSA, HFSW and LFSA foods.

Generally, the finding of the current study highlight that fasting might have a substantial positive impact on health if after fast cognitive controlling of eating is achieved.

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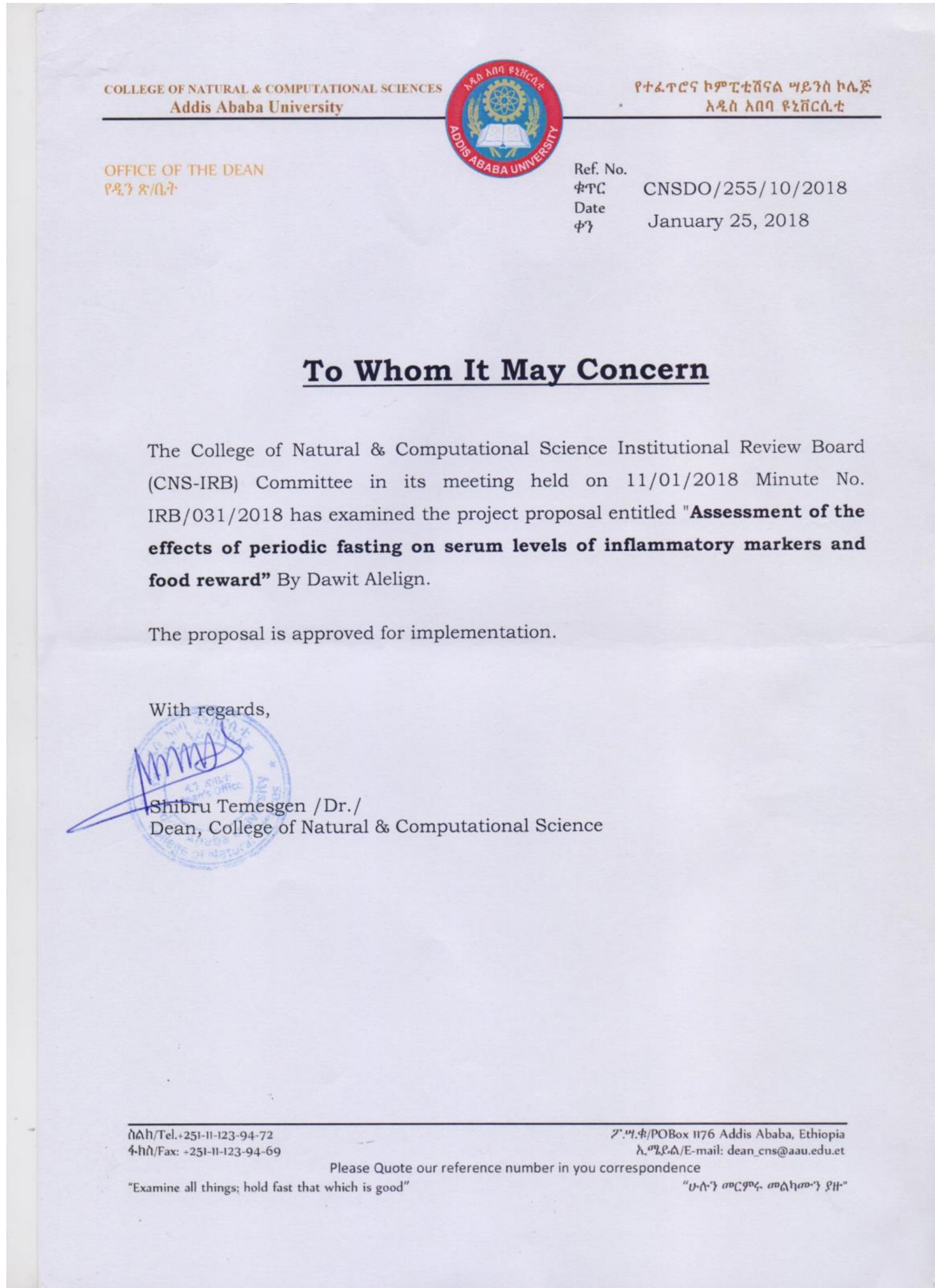
ANNEXES

Annex 1: Letter of Ethical Clearance from College of Natural and Computational Sciences, Addis Ababa University



COLLEGE OF NATURAL & COMPUTATIONAL SCIENCES
Addis Ababa University

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Ref. No.
ቁጥር CNSDO/255/10/2018
Date
ቀን January 25, 2018

To Whom It May Concern

The College of Natural & Computational Science Institutional Review Board (CNS-IRB) Committee in its meeting held on 11/01/2018 Minute No. IRB/031/2018 has examined the project proposal entitled "**Assessment of the effects of periodic fasting on serum levels of inflammatory markers and food reward"** By Dawit Alelign.

The proposal is approved for implementation.

With regards,

Shibru Temesgen /Dr./
Dean, College of Natural & Computational Science

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Please Quote our reference number in you correspondence

"Examine all things; hold fast that which is good"

"ሁሉን መርምሩ መልካሙን ያዙ"

Annex 2: Leeds Food Preference Questionnaire Photographic Stimuli Ratings

Photographs were rated by seventeen adults on three attributes, using a seven-point Likert scale where 1 was the least and 7 was the most(12, 13, 20). The attributes were: typicality, fat content and sweetness. Mean ratings of the sixteen photos used are presented in Table A1. Ratings were used in photograph selection.

Table: A 1 Mean ratings of photographic stimuli used in Leeds Food Preference Questionnaire

Food name	Category	Typicality	Fat content	Sweetness
Cookies	HFSW	7	5.1	6.8
Cream cake	HFSW	6.8	5.3	6.3
Chocolate bar	HFSW	6.7	4.6	6.5
Ice cream	HFSW	6.9	4.8	6.2
Tea with sugar	LFSW	7	1.8	6.6
Strawberries	LFSW	6.8	2.5	5.4
Pineapple	LFSW	6.8	2.3	5.2
Orange	LFSW	7	4.4	6.4
Fried chicken	HFSA	6.8	6	2.7
Burger	HFSA	6.8	5.7	3.5
Doro wot	HFSA	7	5.8	2.3
Kitfo	HFSA	6.8	6.5	2.8
Spaghetti in sauce	LFSA	6.9	3.7	3.2
Lettuce	LFSA	7	2	3
Carrot	LFSA	7	1.7	3.9
Tomato	LFSA	6.2	1.9	2.6

Annex 3: Participant's Information Sheet and Consent Form

A. Information Sheet

Hello, how are you? My name is Dawit Alelign. I am a Masters Student in AAU, college of natural and computational sciences, center for food science and nutrition, as a principal investigator conducting this study. The aim of this study is to assess the effects of periodic fasting on inflammation and food reward with regard to the prevention of cardiovascular and other non-communicable diseases .Your cooperation and willingness to participate in the interview, body measurement &providing 6-10 ml blood sample is very helpful in identifying the effect of fasting on those dependent variables. All comments and responses will be treated confidentially. Your participation is voluntary and you are not obliged to answer any question you do not want to answer. There is no harm if you don't answer the questions .The benefits you get include eating test meal, knowing your blood CRP level and BMI status and getting counseling for improvement of your health status. The interview questions and measurements will take 30- 45 minutes for each test sessions (at baseline or prior to the fasting period and at the end of the fasting period). I would like to interview you few questions regarding socio-demographic and general questions, the Three Factor Eating Questionnaire Revised 18-Item (TFEQ-R18), the Leeds Food Preference Questionnaire (LFPQ) and 24 dietary recalls. Body measurements (height, weight, waist and hip circumference), and blood samples will be collected twice at pre and end of fasting period.

Thank you for helping with this study.

For more information and question here is the contact address of investigator: Dawit Alelign: Mobile: 0912052483; e-mail: dawitalelign6@gmail.com.

B. Consent form

I am informed that the study will be conducted by Masters Student in AAU, college of natural and computational sciences, center for food science and nutrition on assessment of the effects of periodicfasting on inflammation and food reward. The study is aiming to examine the effects of periodic fasting on inflammation and food preferences .This is ultimately important to better understand how fasting can be more effectively used in prevention of cardiovascular and other non-communicable diseases .The participation in the study is entirely voluntary. There is no harm by not answering the questions. The benefits of answering the questions include eating test meal, knowing health status and getting counseling on improvement of health. I heard all the information mentioned above and agree to participate in the study.

Name…………………………………………………………………………….

Signature…………………………………………………………………………

Date…………………………………….

Please return this sheet to the investigator.

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እንደምን ነህ/ሽ?

እኔ ዳዊት አለልኝ እባላለሁ፤

በአዲስ አበባ ዩኒቨርስቲ የተፈጥሮ ሳይንስ በምግብ ሳይንስ ኒውትሪሽን የማስተርስ ደግሪ ለማግኘት የማሟያ የጥናትና የምርምር ስራ እየሠራሁ ነው። ተከታታይ ቀናትን መጾም በሽታ በመከላከል እና የሰውነት ክብደትና ግዝፈት መጠን ልኬት ላይ ያለውን አስተዋጽኦ ለማጥናት በሚደረግው በዚህ ጥናት ተሳታፊ እንዲሆኑ ፍቃድዎን በአክብሮት እጠይቃለሁ። ለጥናቱ የሚኖርዎት ፈቃደኝነትና ቀና ትብብር ለማኅበረሰባችን የአመጋገብ ሥርዓት እና ጤና መካከል በእጅጉ ይጠቅማል።

በመሆኑም እርስዎ በምርምር ስራው ላይ ለመሳተፍ ፍቃደኛ ከሆኑ ለሚጠየቁት ጥያቄ መረጃ በመስጠት፣ የሰውነት ክብደትና ግዝፈት መጠን ልኬት እና የደም ናሙና በመስጠት ትብብር እንዲያደርጉ በትህትና እጠይቃለሁ።

የሚሰጡት ምላሽ ሆነ የደም ናሙና ለጥናትና ምርምር ስራው ውጪ ለሌላ አገልግሎት አይውልም። በመጠየቁ ላይ መመለስ የማይፈልጉት ጥያቄ ካለ አለመመለስ ይችላሉ። በጥናቱ ላይ ተሳትፎ በማድረግዎ ለጥናቱ ስራ የተዘጋጀውን ቁርስ ይመገባሉ፤ የግል የጤና ሁኔታዎን በማወቅ ምክር ያገኛሉ።

ከላይ በተገለጸው መረጃ መሠረት በጥናቱ ለመሳተፍ የተስማሙ መሆኑን ስም በመጻፍና በመፈረም ያረጋግጡ ።

ስም ________________________________

ፊርማ ______________________________

ቀን ______________________

Annex 4: Questionnaire (English Version)

Participant's ID ————————

Date/month/ year of interview ________________ / ______________ / ___________

Time started __________________________Time Ended_____________________

Name _________________________ Mobile ______________________

Physical Address: Sub city ___________ Woreda ____________ H. NO. __________

Respondent's role in the Study

Faster ☐ Non Faster (Control) ☐

I. Questionnaires

1. Socio demographic and general questions

No.	Questions	Responses	Code
1.	Age	________ (full years)	
2.	Sex	1. Male ☐ 2. Female ☐	
3.	Educational Level	1. No formal Education ☐ 2. Primary Level ☐ 3. Secondary Level ☐ 4. Tertiary Level ☐	
4.	Marital Status	1. Single ☐ 2. Married ☐ 3. Divorced ☐ 4. Widowed ☐	

5.	Monthly Income (ETB)	1. No regular income ☐ 2. Less than 999 ☐ 3. From 1,000 to 1,999 ☐ 4. From 2,000 to 2,999 ☐ 5. ≥3,000 ☐	
	For how many years have you participated in fasting periods?	______________________ Year	
6.	During Fasting to what extent do you limit your intake?	1. Depriviation of all ASF + eat break fast ☐ 2. Deprivation of all ASF execpt fish ☐ 3. Deprivation of ASF +skip break fast ☐ 4. Deprivation of ASF + fasting until 3 pm ☐ 5. Other ________________	
7.	What foods do you avoid because of intolerance, food allergy or other reasons except fasting?	List of food _______________________ 1. _________________________ 2. _________________________ 3. _________________________ 4. _________________________	
ASF Animal Source food ETB Ethiopian Birr			

2. The Three-Factor Eating Questionnaire—Revised 18-Item

1. When I smell a sizzling steak or juicy piece of meat, I find it very difficult to keep from eating, even if I have just finished a meal.

   Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

2. I deliberately take small helpings as a means of controlling my weight.

   Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

3. When I feel anxious, I find myself eating.

   Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

4. Sometimes when I start eating, I just can't seem to stop.

   Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

5. Being with someone who is eating often makes me hungry enough to eat also.

   Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

6. When I feel blue, I often overeat.

   Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

7. When I see a real delicacy, I often get so hungry that I have to eat right away.

   Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

8. I get so hungry that my stomach often seems like a bottomless pit.

   Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

9. I am always hungry so it is hard for me to stop eating before I finish the food on my plate.

   Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

10. When I feel lonely, I console myself by eating.

    Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

11. I consciously hold back at meals in order not to weight gain.

    Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

12. I do not eat some foods because they make me fat.

    Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

13. I am always hungry enough to eat at any time.

    Definitely true (4)/ mostly true (3)/ mostly false (2)/ definitely false (1)

14. How often do you feel hungry?

Only at meal times (1)/ sometimes between meals (2)/ often between meals (3)/ almost always (4)

15. How frequently do you avoid “stocking up” on tempting foods?

    Almost never (1)/ seldom (2)/ usually (3)/ almost always (4)

16. How likely are you to consciously eat less than you want?

    Unlikely (1)/ slightly likely (2)/ moderately likely (3)/ very likely (4)

17. Do you go on eating binges though you are not hungry?

    Never (1)/ rarely (2)/ sometimes (3)/ at least once a week (4)

18. On a scale of 1 to 8, where 1 means no restraint in eating (eating whatever you want, whenever you want it) and 8 means total restraint (constantly limiting food intake and never “giving in”), what number would you give yourself?

    The 1–2 scores were coded 1; 3–4 scores were coded 2; 5–6 scores were coded 3; 7–8 scores were coded 4.

The cognitive restraint scale was composed of items 2, 11, 12, 15, 16, and 18. The uncontrolled eating scale was composed of items 1, 4, 5, 7, 8, 9, 13, 14, and 17. The emotional eating scale was composed of items 3, 6, and 10

3. Leeds Food Preference Questionnaire

Please answer the following questions by placing a vertical mark through the line according to the photographic stimuli.

A. How pleasant would it be to taste some of this food now?

1. Not at all____Extremely
2. Not at all____Extremely
3. Not at all____Extremely
4. Not at all____Extremely
5. Not at all____Extremely
6. Not at all____Extremely
7. Not at all____Extremely
8. Not at all____Extremely
9. Not at all____Extremely
10. Not at all____Extremely
11. Not at all____Extremely
12. Not at all____Extremely
13. Not at all____Extremely
14. Not at all____Extremely
15. Not at all____Extremely
16. Not at all____Extremely

B.How much would you like to eat some of this food now?

1. Not at all____ Extremely
2. Not at all____ Extremely
3. Not at all____ Extremely
4. Not at all____ Extremely
5. Not at all____ Extremely
6. Not at all____ Extremely
7. Not at all____ Extremely
8. Not at all____ Extremely
9. Not at all____ Extremely
10. Not at all____ Extremely
11. Not at all____ Extremely
12. Not at all____ Extremely
13. Not at all____ Extremely
14. Not at all____ Extremely
15. Not at all____ Extremely
16. Not at all____ Extremely

4. Twenty four hour dietary recall

Participant’s Code_______________

Please tell me everything that you ate and drank yesterday.

A. Quick List

Breakfast	Snack	Lunch	Snack	Dinner	Snack

B. Detailed list

Participant's Code_______________

Measurements taken in the home			office	Measurements taken in the home						Net grams (office)			Is the meal has ingredient (0=no,1= yes) If the answer is 1= go to next page or table
15. Meal-time	Name of food or dish	Ingredients and characteristics	16. Code of food or dish	Served (gm/ml)	Not Consumed	Consumed	Weighed (1=gross 2=net)	How consumed (1=cooked 2=raw)	17. Days consumed in past week	Conversion to grams	18. Served	19. Consumed	

Mealtime:

Meals code: 10 mornings (breakfast); 20 mid-day (lunch); 30 evening (dinner)

Snacks: 11, 12, 13 ... 19 Morning (after breakfast); 21, 22, 23 … 29 Afternoon (After lunch);31, 32, 33 … 39 Evening (after dinner)

C. If the food has ingredients

Participant's Code________________

Measurements taken in the home				Office		Measurements taken in the home				Office	
Dish:				Total weight of cooked ingredients:		Dish:				Total weight of cooked ingredientsused for child	
Ingredients	Quantity used (gm/ml)	Weighed (1=gross 2=net)	Used (1=cooked 2=raw)	Conversion to cooked	Cooked grams	Ingredients	Quantity used (gm/ml)	Weighed (1=gross 2=net)	Used (1=cooked 2=raw)	Conversion to cooked	Cooked grams

II. Anthropometric and hs-CRP measurements

No.	Variables/ parameters	Pre-fasting	Post fasting	Difference
1.	Weight (KG)			
2.	Height (CM)			
3.	Waist Circumference (CM)			
4.	Hip Circumference (Cm)			
5.	BMI (Kg/m^2)			
6.	Waist-Hip Ratio			
7.	hs-CRP			

hs-CRP, High sensitivity C -reactive protein

Annex -5: Questionnaires – Amharic version (መጠይቅ)

የጥናቱ ተሳታፊ መለያ ኮድ ________________________

ቃለ-መጠይቁ የተደረገበት ቀን __________/___________/ 2010 ዓ.ም

ቃለ-መጠይቁ የጀመረበት ሰዓት ______________ ያበቃበት ሰዓት ____________________

የተጠያቂ ስም ______________________ የሞባይል ስልክ ______________________

አድራሻ ክ/ከተማ ____________________ ወረዳ ______________ የቤት ቁ. __________

ግለሰቡ/ቧ በጥናቱ ውስጥ የሚኖራቸው ሚና፤ ጿሚ ☐ የማይፆሙ ☐

I.ቃለመጠይቅ

1. የግለሰቡ/ቧ ማህበራዊ፣ ሥነ-ህዝባዊ እና ኢኮኖሚያዊ እንዲሁም የአመጋገብ ስርዓት መጠይቅ

ተ.ቁ	ጥያቄዎች	ምላሾች	ኮድ
1.	ዕድሜ	________________________	
2.	ፆታ	1. ወንድ ☐ 2. ሴት ☐	
3.	የትምህርት ደረጃ	1. መደበኛ ትምህርት ያልተማሩ☐ 2. መጀመሪያ ደረጃ ☐ 3. ሁለተኛ ደረጃ ☐ 4. ከሁለተኛ ደረጃ በላይ☐	
4.	የጋብቻ ሁኔታ	1. ያላገቡ ☐ 2. ያገቡ ☐ 3. የተፋቱ ☐ 4. የትዳርን አጋራቸውን በሞት ያጡ ☐	
5.	ወርሃዊ ገቢ (ብር)	1. መደበኛ የገቢ ምንጭ የሌላቸው ☐ 2. ከ999 ብር ያነሰ ☐ 3. ከ1,000 እስከ 1,999 ብር ☐ 4. ከ2,000 እስከ 2,999 ብር ☐ 5. 3,000 ብር እና ከዚያ በላይ ☐	
6.	መጾም ከጀመሩ ምን ያህል ዓመታት ሆኗል?	________________________ ዓመታት	

7.	በፆም ወቅት የሚከተሉትን የአመጋገብ ሥርዓት ቢገልፁ?	1. ሁሉንም የእንስሳት ተዋህፆ አልመገብም፤ በተጨማሪም፤ ቁርስ እመገባለሁ ☐ 2. ሁሉንም የእንስሳት ተዋህፆ ከዓሣ ውጪ አልመገብም☐ 3. ከሁሉም የእንስሳት ተዋህፆ በተጨማሪም ቁርስ አልመገብም ☐ 4. ከሁሉም የእንስሳት ተዋህፆ በተጨማሪም እስከ ቀኑ 9፡00 ሰዓት አልመገብም☐ 5. ሌላ ________________	
8.	ሲመገቡ የማያስማማዎት ወይም አለርጂ የሚያመጣብዎ የምግብ ዓይነት ካለ፤ በዚህም ምክንያት ፈጽሞ የማይመገቡት ከሆነ የምግቡን ዓይነት ወይም ስም ቢገልጹ?	____________________ ____________________ ____________________ ____________________	

2. (ዘ ስሪ ፋክተር የአመጋገብ ባህሪ ልኬት መጠይቅ)**The Three-Factor Eating Questionnaire—Revised 18-Item**

1. በሚያምር ሁኔታ የተጠበሰና ሽታው የሚያስጎመዥ ስጋ ጥብስ ሳይ ምግብ ተመግቤ መነሳቴ ቢሆን እንኳ አልበላም ብዬ ራሴን መገደብ ይከብደኛል፡፡

☐ ሁልጊዜ ☐ ባብዛኛው ☐ የተወሰነ ጊዜያት ☐ በጭራሽ

2. ውፍረቴን ለመቆጣጠር ምግብ ስመገብ ጥቂት እወስዳለሁ፡፡

☐ ሁልጊዜ ☐ ባብዛኛው ☐ የተወሰነ ጊዜያት ☐ በጭራሽ

3. የጭንቀት ስሜት ሲሰማኝ ምግብ በብዛት እመገባለሁ፡፡

☐ ሁልጊዜ ☐ ባብዛኛው ☐ የተወሰነ ጊዜያት ☐ በጭራሽ

4. አንዳንድ ጊዜ መብላት ከጀመርኩ ማቆም ይከብደኛል፡፡

☐ ሁልጊዜ ☐ ባብዛኛው ☐ የተወሰነ ጊዜያት ☐ በጭራሽ

5. በጥሩ ሁኔታ ከሚመገብ ሰው ጋር ስሆን እኔም የመብላት ፍላጎቴ ይጨምራል፡፡

☐ ሁልጊዜ ☐ ባብዛኛው ☐ የተወሰነ ጊዜያት ☐ በጭራሽ

6. ውስጤን ሲከፋኝ አብዝቼ እመገባለሁ፡፡

☐ ሁልጊዜ ☐ ባብዛኛው ☐ የተወሰነ ጊዜያት ☐ በጭራሽ

7. በጥሩ ሁኔታ የተሰናዳ ምግብ ሳይ ወዲያውኑ የረሃብ ስሜቴ ይቀሰቀስና መብላት ያምረኛል፡፡

☐ ሁልጊዜ ☐ ባብዛኛው ☐ የተወሰነ ጊዜያት ☐ በጭራሽ

8. ሁልጊዜ የረሃብ ስሜት ስለሚሰማኝ በልቼ የማልጠግብ ያህል ይሰማኛል፡፡

☐ ሁልጊዜ ☐ ባብዛኛው ☐ የተወሰነ ጊዜያት ☐ በጭራሽ

9.ከፍተኛ የሆነ የረሃብ ስሜት ስለሚሰማኝ ከፊት ሳህኔ ላይ የቀረበውን ምግብ ሳልጨርስ ማቆም ይከብደኛል፡፡

☐ ሁልጊዜ ☐ ባብዛኛው ☐ የተወሰነ ጊዜያት ☐ በጭራሽ

10.ብቸኝነት ሲሰማኝ ብዙ ምግብ በመመገብ እራሴን ዘና ለማድረግ እሞክራለሁ፡፡

☐ ሁልጊዜ ☐ ባብዛኛው ☐ የተወሰነ ጊዜያት ☐ በጭራሽ

11.ክብደት ላለመጨመር በሚል ፍራቻ ምግቦችን እምቢ እላለሁ፡፡

☐ ሁልጊዜ ☐ ባብዛኛው ☐ የተወሰነ ጊዜያት ☐ በጭራሽ

12.የተወሰኑ የምግብ አይነቶች ስለሚያወፍሩኝ አልመገባቸውም፡፡

☐ ሁልጊዜ ☐ ባብዛኛው ☐ የተወሰነ ጊዜያት ☐ በጭራሽ

13.በየትኛውም ጊዜ የረሃብ ስሜት ስለሚሰማኝ መመገብ እችላለሁ፡፡

☐ ሁልጊዜ ☐ ባብዛኛው ☐ የተወሰነ ጊዜያት ☐ በጭራሽ

14.የረሃብ ስሜት በምን ያህል ጊዜ ይሰማሃል/ሻል?

☐ በመመገቢያ ሰዓት ብቻ (ምሳ፣ ቁርስ፣ራት) ☐ በመመገቢያ ሰዓት መሀል መሀል ላይ በአብዛኛው

☐ በመመገቢያ ሰዓት መሀል ላይ አንዳንዴ ☐ ሁል ጊዜ ይርበኛል፡፡

15.የሚያስጎመጁ ምግቦችን መግዛት ለማቆም ምን ያህል ተደጋጋሚ ጥረት አድርገሃል?

☐ በፍጹም አላደርገውም ማለት ይቻላል ☐ ባብዛኛው ጊዜ ሞክራለሁ

☐ አልፎ አልፎ ሞክራለሁ ☐ ሁልጊዜ ሞክራለሁ

16.በማስተዋል ሆን ብለህ/ሽ መብላት ከምትፈልገው/ጊው በታች የምትመገብበት/ቢበት አጋጣሚዎች ምን ያህል ናቸው?

☐ የሉም ☐ የተወሰኑ አሉ ☐ በአብዛኛው አሉ ☐ በጣም ብዙ ጊዜ አሉ

17.የረሃብ ስሜት ሳይሰማህ/ሽ ከመጠን በላይ ምግብ ለመመገብ የምትነሳሳበት/ሺበት/ አጋጣሚ

☐ የሉም ☐ በጣም በጥቂቱ አለ ☐ አልፎ አልፎ አለ ☐ ቢያንስ በሳምንት አንድ ጊዜ

18.ከ1-8 ባሉት ቁጥሮች 1 በየትኛውም ምግብ ላይ ክልከላ ሳይደረግ መብላት እና የፈለጉትን መመገብ ሆኖ 8 ደግሞ የተመረጡ ምግቦችን መመገብ እና እራስን መቆጣጠር ቢወክል ለራስህ/ሽ ስንት ቁጥርን ትሰጣለህ/ጫለሽ??

የምክንያታዊ መከላከል ልኬታዎች በቁጥር 2፣11፣12፣15፣16፣18 የተገለፁት ሲሆኑ ያልተገደበ የምግብ አወሳሰድ ልኬታዎች በቁጥር 1፣4፣5፣7፣8፣9፣13፣14፣17 ላይ ተገልፀዋል እንዲሁም በስሜቶች ላይ የተመሰረተ የአመጋገብ መለኪያዎችን በተራ ቁጥር 3፣6 እና 10 ተገልፀዋል።

3. የሊድስ የምግብ ምርጫ ልኬት መጠይቅ

/Leads food preference Questionnaire/

እባክዎ ከታች የቀረቡትን ጥያቄዎች በሚያዩት የምግብ ፎቶዎች መሰረት በመስመሩ ላይ በማስመር መልሱ።

ሀ. አሁን ምግቡን ማጣጣም ምን ያህል አስደሳች ነው?

1. በጭራሽ አያስደስትም ———————————————— እጅግ በጣም ያስደስታል
2. በጭራሽ አያስደስትም ———————————————— እጅግ በጣም ያስደስታል
3. በጭራሽ አያስደስትም ———————————————— እጅግ በጣም ያስደስታል
4. በጭራሽ አያስደስትም ———————————————— እጅግ በጣም ያስደስታል
5. በጭራሽ አያስደስትም ———————————————— እጅግ በጣም ያስደስታል
6. በጭራሽ አያስደስትም ———————————————— እጅግ በጣም ያስደስታል
7. በጭራሽ አያስደስትም ———————————————— እጅግ በጣም ያስደስታል
8. በጭራሽ አያስደስትም ———————————————— እጅግ በጣም ያስደስታል
9. በጭራሽ አያስደስትም ———————————————— እጅግ በጣም ያስደስታል
10. በጭራሽ አያስደስትም ———————————————— እጅግ በጣም ያስደስታል
11. በጭራሽ አያስደስትም ———————————————— እጅግ በጣም ያስደስታል
12. በጭራሽ አያስደስትም ———————————————— እጅግ በጣም ያስደስታል
13. በጭራሽ አያስደስትም ———————————————— እጅግ በጣም ያስደስታል
14. በጭራሽ አያስደስትም ———————————————— እጅግ በጣም ያስደስታል
15. በጭራሽ አያስደስትም ———————————————— እጅግ በጣም ያስደስታል
16. በጭራሽ አያስደስትም ———————————————— እጅግ በጣም ያስደስታል

ለ. ምግቡን አሁን ምን ያህል መጠን መመገብ ትፈልጋለህ?

1. በጭራሽ አልፈልግም ———————————————— እጅግ በጣም ብዙ መጠን እፈልጋለሁ
2. በጭራሽ አልፈልግም ———————————————— እጅግ በጣም ብዙ መጠን እፈልጋለሁ
3. በጭራሽ አልፈልግም ———————————————— እጅግ በጣም ብዙ መጠን እፈልጋለሁ
4. በጭራሽ አልፈልግም ———————————————— እጅግ በጣም ብዙ መጠን እፈልጋለሁ
5. በጭራሽ አልፈልግም ———————————————— እጅግ በጣም ብዙ መጠን እፈልጋለሁ
6. በጭራሽ አልፈልግም ———————————————— እጅግ በጣም ብዙ መጠን እፈልጋለሁ
7. በጭራሽ አልፈልግም ———————————————— እጅግ በጣም ብዙ መጠን እፈልጋለሁ
8. በጭራሽ አልፈልግም ———————————————— እጅግ በጣም ብዙ መጠን እፈልጋለሁ
9. በጭራሽ አልፈልግም ———————————————— እጅግ በጣም ብዙ መጠን እፈልጋለሁ
10. በጭራሽ አልፈልግም ———————————————— እጅግ በጣም ብዙ መጠን እፈልጋለሁ
11. በጭራሽ አልፈልግም ———————————————— እጅግ በጣም ብዙ መጠን እፈልጋለሁ
12. በጭራሽ አልፈልግም ———————————————— እጅግ በጣም ብዙ መጠን እፈልጋለሁ
13. በጭራሽ አልፈልግም ———————————————— እጅግ በጣም ብዙ መጠን እፈልጋለሁ
14. በጭራሽ አልፈልግም ———————————————— እጅግ በጣም ብዙ መጠን እፈልጋለሁ
15. በጭራሽ አልፈልግም ———————————————— እጅግ በጣም ብዙ መጠን እፈልጋለሁ
16. በጭራሽ አልፈልግም ———————————————— እጅግ በጣም ብዙ መጠን እፈልጋለሁ

4. የ24 ሰዓት ውስጥ የአመጋገብ መጠይቅ

ኮድ:___________________

እባክዎን በትላትናዉ እለት የበሉትን እና የጠጡትን ይንገሩን

1. ፈጣን ምላሽ

ቁርስ	መቆያ	ምሳ	መቆያ	እራት	መቆያ

2. ሰፊ ዝርዝር

ኮድ:_________________

ቤት ዉስጥ የተወሰደ ልኬት			ቢሮ	ቤት ዉስጥ የተወሰደ ልኬት						የተጣራ ግራም (ቢሮ)			በምግቡ ውስጥ ያሉት ግብዓቶች መዘርዝር ይፈልጋል (0 = የለም፣ 1 = አዎ) መልሱ 1 ከዎነ ወደ ሚቀው ገጽ ወይም ሰንጠረዝ ይሂዱ
15. የምግብ ሰዓት	የምግቡ ስም	የተሰራበት ንጥረ ምግብ እና ባህሪያቶቹ	16. የምግቡ ኮድ	የቀረበው ምግብ መጠን	ያልተመገበዉ ምግብ መጠን(የቀረ)	የተመገበዉ ምግብ መጠን	ክብደት 1= አጠቃላይ 2=የተጣራ	የአመጋገብ ሁኔታ 1=የበሰለ 2=ጥሬ	17.ባለፈዉ ሳምንት የተመገባቸዉ ቀናት	ወደ ግራም ሲለወጥ	18. የቀረበው ምግብ መጠን	19. የተመገበዉ ምግብ መጠን	

የምግብ ሰዓት፣ ዋና ዋና ምግቦች 10 ጧት (ቁርስ); 20 እኩለ ቀን (ምሳ); 30 ማታ (እራት)
መክሰሶች: 11, 12, 13 ... 19 ጧት (ከቁርስ በኋላ); 21, 22, 23 … 29 ከሰዓት(ከምሳ በኋላ); 31, 32, 33 … 39 ምሽት(ከእራት በኋላ)

3 ኛ ምግቡ ግብዓቶች ካሉት

ቤት ዉስጥ የተወሰደ ልኬት				ቢሮ		ቤት ዉስጥ የተወሰደ ልኬት				ቢሮ/ መስክ	
የምግቡ ስም				አጠቃላይ የበሰለዉ		የምግቡ ስም				አጠቃላይ ሕጻኑ የተጠቀመዉ	
ግብዓቶች	የተጠቀመዉ መጠን	ክብደት (1=አጠቃላይ 2=የተጣራ)	የተመገበው (1=የበሰለ	ወደ በሰለዉ ሲለወጥ	የበሰለዉ በግራም	ግብዓቶች	የተጠቀመዉ ብዛት	ክብደት (1= አጠቃላይ 2=የተጣራ)	የተጠቀመዉ (1=የበሰለ	ወደ በሰለዉ ሲለወጥ	የበሰለዉ በግራም

II.የሰውነት ክብደትና ግዝፈት መጠንእና የደም ናሙና ልኬት (Anthropometric and hs-CRP measurements)

No.	Variables/ parameters	Pre-fasting	Post fasting	Difference
8.	Weight (KG)			
9.	Height (CM)			
10.	Waist Circumference (CM)			
11.	Hip Circumference (Cm)			
12.	BMI (Kg/m^2)			
13.	Waist-Hip Ratio			
14.	hs-CRP			

hs-CRP,High sensitivity C -reactive protein

Annex 6: DECLARATION

I, the undersigned, declare that this thesis is my original work and that all sources of materials used for the thesis have been fully acknowledged.

Name: Dawit Alelign

Signature: ______________________ Date: _________________________

This thesis work has been submitted for examination with my approval as university advisor.

Name of Advisor	Signature	Date
Dr. Kaleab Baye	----------------------------	--------------------------