Appendices

APPENDIX – I

Ethical Committee Clearance

INSTITUTIONAL HUMAN ETHICS COMMITTEE

Avinashilingam

Institute for Home Science and Higher Education for Women

Deemed to be University Under category 'A' By MHRD, (Estd. u/s 3 of UGC Act 1956)
Re Accredited with 'A' Grade By NAAC, Recognised by UGC Under Section 12 B
Coimbatore - 641043, Tamil Nadu, India

Seek and Ye Shall Find

Chairman
Dr. S. Ramalingam
Principal, PSG Institute
of Medical Sciences
& Research, Coimbatore

Member Secretary
Dr.S.Uma Mageshwari
Professor,
Dean Student Affairs,
Department of Food Service
Management & Dietetics

Members
Dr.P.R.Padma
Mr. K.Arulmoli (Legal Expert)
Dr. N.S. Rohini
Dr.Subhashini K. Sripathi
Dr.A. Saraswathy
Ms.D.Kavitha
Dr.S. Muthulakshmi
Dr.G.Victoria Naomi
Dr. Judith Justin
Dr.Anitha Subash

3rd June 2019

To
Ms. Thamilovia.SA
Department of Food Service Management and Dietetics
Avinashilingam Institute for Home Science and
Higher Education for Women
Coimbatore – 641 043

Dear Thamilovia.SA

Ref: Your presentation of the proposal No. IHEC/18-19/FSMD/26 entitled "Association of Visceral Adiposity with Insulin Resistance among Young Obese Women" to the IHEC on 24th May 2019

The Institutional Human Ethics Committee of our University hereby grants approval to your research proposal No. IHEC/18-19/FSMD/26 entitled "Association of Visceral Adiposity with Insulin Resistance among Young Obese Women" submitted and presented by you. The Approval number for the same is AUW/IHEC-18-19/FSMD/FHP-01.

We wish you all the best in your research endeavours.

Regards,

V. Uma Mageshwari
Dr.S.Uma Mageshwari
Member Secretary

Institutional Human Ethics Committee

APPENDIX – II

Consent Form

INFORMED CONSENT FORMAT FOR RESEARCH PROJECTS

(Strike off items that are not applicable)

I, SA. Thamilovia, am carrying out a study on the topic "**Association of Visceral Adiposity Index and Lipid Accumulation Product with Insulin Resistance among Selected Adult Women and the Impact of Intervention"** as part of my research project being carried out under the aegis of the Department of Food Service Management and Dietetics

My research guide is: Dr. S. Uma Mageshwari
(Applicable to students only)

The justification for this study is:

Studies related to visceral adiposity and insulin sensitivity are minimal carried out in India. By doing this research, the prevalence of obesity and diabetes can be found and appropriate interventional strategies can be used to bring down the occurrence rate. There is need for evidence-based studies to find out the association of adiposity, insulin resistance and depression so that intervention can be directed towards decreasing visceral adiposity rather than total weight management. This proposed study will be of interest to examine the association between abdominal adiposity and depression among the population along with its relation with insulin resistance.

The objectives of this study are:
Primary Objective(s):

- Find the prevalence of obesity among the adult women.
- Associate and correlate visceral adiposity index, lipid accumulation product and insulin resistance among experimental and control groups.
- Find the risk factors and cut-off points associated with Visceral Adiposity Index and Lipid Accumulation Product

Secondary Objective(s):

- Apply and validate visceral adiposity index and lipid accumulation cut-off points among adult women
- Study the dietary pattern among experimental and control groups.
- Assess the physical activity pattern among experimental and control groups.
- Study the anthropogens among experimental and control groups.
- Impart intervention for obesity management and evaluate the impact of the intervention on VAI and LAP.

Sample size: 970

Study volunteers / participants are (specify population group & age group): Adult Women of age group 18-30 years

Location of the study: Coimbatore

We request you to kindly cooperate with us in this study. We propose collect background information and other relevant details related to this study. We will be carrying out:

Initial interview (specify approximate duration): 20 minutes.

Data collected will be stored for a period of fifteen years. We will / will not use the data as part of another study.

Health education sessions: Number of sessions: 2

Approximate duration of each session: 30 minutes.

Clinical examination (Specify details and purpose): NA

Blood sample collection: Specify quantity of blood being drawn: 5 ml.

No. of times it will be collected: twice (pre and post evaluation)

Whether blood sample collection is part of routine procedure or for research (study purpose):

☐ Routine Procedure ☑ Research Purpose

Specify purpose, discomfort likely to be felt and side effects, if any: NA

Will the blood sample collected be stored after study period: ☐ Yes
☑ No, it will be destroyed

Will the blood sample collected be sold: ☐ Yes ☑ No

Will the sample collected be shared with persons from another institution: ☐ Yes ☑ No

Medication / supplementation given, if any, with duration, side effects, purpose, benefits:

Is the medication / supplementation given part of routine procedure: ☐ Yes ☐ No
(If no, state reasons for giving this medication/supplementation)

NA

Are alternatives available for medication / supplementation given: ☐ Yes ☐ No
(If no, state reasons for giving this particular medication/supplementation)

NA

Final interview (specify approximate duration): 30 minutes.

If photograph is taken, purpose: For the purpose of writing thesis

Benefits from this study, if any :
The research outcome of the project is

- The prevalence of obesity among population will be identified.
- Biomarkers for Visceral Adiposity Index, Insulin Resistance among the selected women can be obtained.
- Derive the cut-off points of visceral adiposity index and lipid accumulation product
- Depending the derived cut-off, the prevalence of visceral adiposity will be found.
- Effectiveness of custom-made interventions particularly effect of yoga on visceral adiposity will be analysed.

Risks involved by participating in this study, if any : NA

How will the results be used?

The results of this proposed study will be used to educate the community on visceral adiposity and importance of physical activity and balanced diet.

If you are uncomfortable in answering any of our questions during the course of the interview / biological sample collection, you have the right to withdraw from the interview / study at any time. You have the freedom to withdraw from the study at any point of time. You will NOT be paid any remuneration for the time you spend with us for this interview / study. The information provided by you will be kept in strict confidence. Under no circumstances shall we reveal the identity of the respondent or their families to anyone. The information that we collect shall be used for approved research purposes only. You will be informed about any significant new findings – including adverse events, if any – whether directly or indirectly related to you or to other participants of this study, developed during the course of this research which may relate to your willingness to continue participation

Consent: The above information regarding the study, has been read by me, and has been explained to me by the investigator(s). Having understood the same, I hereby give my consent to them to interview me, and collect biological sample 5ml from me. I am affixing my signature / left thumb impression to indicate my consent and willingness to participate in this study (i.e., willingly abide by the project requirements)

Signature / Left thumb impression of the Study Volunteer / Legal Representative:

Signature of the Interviewer with date

Signature of the Witness with name:

APPENDIX – III

Sample Size Calculation

The sample size was calculated keeping the overall prevalence of abdominal obesity given by Indian Council of Medical Research – India Diabetes (ICMR-INDIAB, 2015) study. It was a cross-sectional National study on the prevalence of diabetes and related disorders such as obesity and hypertension, funded by the ICMR and the Department of Health Research (DHR), Government of India. The study sampled all the 28 States (now 29 States after the State of Andhra Pradesh was divided into Telangana and Andhra Pradesh) in India. The prevalence of abdominal obesity (AO) was 32.3% among women in Tamil Nadu (Pradeepa et al., 2015). Using the formula given below, the effect size was determined to estimate the sample size as the denominator.

Effect size (ES)

$$ES = \frac{p1-p0}{\sqrt{p0(1-p0)}} = \frac{0.05}{\sqrt{0.323(1-.323)}}$$

$$= \frac{0.05}{\sqrt{0.323\,(0.677)}} = \frac{0.05}{\sqrt{0.219}} = \frac{0.05}{0.468}.$$

$$= 0.11$$

$$N = \left[\frac{Z1-\alpha/2+Z1-\beta}{ES}\right]^2 = \left[\frac{1.96+1.282}{0.11}\right]^2 = \left[\frac{3.242}{0.11}\right]^2$$

$$= (29.47)^2 = \mathbf{868.48}$$

APPENDIX – IV

Elicit Background Information Questionnaire

Socio-economic Background

1. Name :
2. Age :
3. Date of birth :
4. E-mail address :
5. Contact Number :
6. Qualification

   ☐ B.Sc. ☐ M.Sc. ☐ M.Phil./Ph.D.

7. Class and Department _____
8. Name of the Institution now studying in

   _____
9. Occupation of the parents

   Father _____

   Mother _____
10. Family Income

    Monthly income in rupees

    ☐ Less than 7300 ☐ 7300 to 14500 ☐ Above 14500
11. Marital Status

    ☐ Single ☐ Married

Anthropometric Measurements

12. Height (cm) :
13. Weight (kg) :
14. BMI :
15. Waist Circumference (cm) :
16. Hip Circumference (cm) :
17. Waist Hip Ratio (WHR) :
18. Waist Height Ratio (WHtR) :
19. Blood pressure (mm/Hg) :

Biochemical Profile

20. Lipid profile

a) Total cholesterol
b) Triglycerides
c) HDL cholesterol
d) LDL cholesterol

21. Blood sugar profile

a) Fasting blood sugar
b) Fasting insulin

APPENDIX – V

Total Cholesterol (CHOD/POD Method)

Cholesterol is a fat like substance that is found in all body cell. The liver makes all the cholesterol the body needs to form cell membranes and to make certain hormones. The determination of Serum Cholesterol is one of the important tools in the diagnosis and classification of lipemia. High blood cholesterol is one of the major risk factor of heart disease. Clinical Diagnostic should not be made on a single test result. It should integrate clinical and other laboratory data.

Principle:

Cholesterol esters in Serum are hydrolysed by cholesterol esterase (CHE). The free cholesterol produced is oxidized by cholesterol oxidase (Co) to form Cholest 4en- 3-one with simultaneous production of hydrogen peroxide (H2O2) which oxidatively couples with 4-aminoantipyrine and phenol in the presence of peroxidase (POD) to yield a red chromophore.

Cholesterol ester + H_2O $\xrightarrow{CHE}$ Cholesterol + Fatty acid

Cholesterol + O_2 $\xrightarrow{CHOD}$ Cholesterol-3-one + H_2O_2

H_2O_2 + 4-AAP + Phenol $\xrightarrow{POD}$ Red Quinoneimine + H_2O

The red Quinoneimine dye formed is measured spectrophotometrically at 505 nm as an increase in absorbance.

Reagents:

Each Cholesterol kit contains

R: Enzyme Reagent

Cholesterol Standard: 200mg/dL

Reagent Preparation

Reagent 1 is ready for use

Stability:

All the components of the kit are stable until the expiration date on the label when stored at 2–8 °C, protected from light and contamination prevented during their use. Do not use reagent over the expiry date. Reagent may develop a slight pink color which does

not affect results provided the blank is run with every batch of tests and a standard included periodically.

Cholesterol STD:

Stable up to the expiry when stored tightly closed at 2–8 °C. Protected from light and contamination prevented during their use.

Signs of reagent deterioration:

- Presence of particles and turbidity
- Blank Absorbance (A) at 505 nm > 0.160
- Standard reading is abnormally low.

Procedure:

1. Assay conditions: Wavelength: 505 nm
   Cuvette: 1 cm light path
   Constant temperature 37ºC

In three colorimetric test tubes labelled B (Blank), S (Standard) and U (Unknown Sample) place

	Blank(B)	**Standard(S)**	**Unknown Samples(U)**
Reagent	1000µL	1000µL	1000µL
Distilled Water	10µL	-	-
Standard	-	10µL	-
Sample	-	-	10µL

2. Mix thoroughly.

3. Incubate for 5 minutes at 37 °C or 15 min. at Room Temperature (15 -25°C).

4. Read in Spectrophotometer at 505 nm or in photo colorimeter with green filter (490-530nm) setting instrument to zero with reagent blank.

Calculation:

$$\text{Cholesterol(mg/dl)} = \frac{\text{(A)Sample}}{\text{(B) Standard}} \times 200 \text{ (standard concentration)}$$

Reference Range

Risk Evaluation:

Less than 200 mg/dL	Normal/Recommended
200 – 240 mg/dl	Borderline
>240 mg/dL	High Risk

Notes

1. Use clean disposable pipette tips for dispensation

Only for *in vitro* use in Clinical laboratory (IVD)

Reference:

Trinder P. Ann. Clin. Biochem, 6: 24 (1969)

APPENDIX - VI

Triglycerides (GPO-PAP Method)

The triglycerides (GPO) method is based on the enzymatic determination of glycerol using the enzyme glycerol phosphate oxidase (GPO) after hydrolysis by lipoprotein lipase. The principle of this method was described by Fossati1 who coupled the reaction with the classical Trinder2 reaction sequence. This single reagent procedure quantitates the total glycerides in serum including the mono and triglycerides, and the free glycerol fractions. This approach is the basis for this method.

Principle:

$$\text{Triglycerides} \xrightarrow{\text{Lipase}} \text{Glycerol + Fatty Acids}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{GK}} \text{Glycerol-1-phosphate + ADP}$$

$$\text{Glycerol - 1 - phosphate + O2} \xrightarrow{\text{GPO}} \text{DAP + H2O2}$$

$$\text{H2O2 + 4-AA +4 -Chlorophenol} \xrightarrow{\text{POD}} \text{Quinoneimine Dye + HCL + 2H2O}$$

Serum triglycerides are hydrolyzed to glycerol and free fatty acids by lipase. In the presence of ATP and glycerol kinase (GK), the glycerol is converted to glycerol-1-phosphate. The glycerol-1-phosphate is then oxidized by glycerol phosphate oxidase (GPO) to yield hydrogen peroxide. The condensation of hydrogen peroxide with 4-chlorophenol and 4-aminophenazone (4-AA) in the presence of peroxidase (POD) produces a red coloured quinonimine dye which absorbs at, or near 500nm. The intensity of the coloured complex formed is directly proportional to the triglyceride concentration of the sample.

Materials Provided:

Triglycerides (GPO) reagent

Materials Required but not Provided:

1. Accurate pipetting devices for delivering required sample and reagent volumes.
2. Test Tubes and racks
3. Timer
4. Heating block or water bath (37°C)

5. Spectrophotometer able to read at 500 nm
6. Triglycerides standard or calibrator

Procedure (Automated-General):

Wavelength	: 500nm
Assay Type	: Endpoint Sample/Reagent
Ratio	: 1:101
Reaction Direction	: Increasing
Temperature	: 37°C Incubation
Time	: 300 sec.
Low Normal	: 44mg/dl
High Normal	: 148mg/dl

Test Procedure (Manual)

1. Label test tubes: “Blank”, “Calibrator/Standard”, “Patient”, “Control”, etc.
2. Pipette 1.0 ml of reagent into the appropriate tubes and pre-warm to 37°C.
3. Add 0.010ml (10ul) of the appropriate sample to their respective tubes. Swirl gently to mix.
4. Incubate all tubes for five (5) minutes.
5. After incubation, zero the spectrophotometer with “Blank” tube, at 500nm. (500-520 nm is acceptable).
6. Read and record the absorbance (Abs.) of all the tubes. The final color is stable for at least 60 minutes.

Limitations:

The procedure is linear to 1000 mg/dl (11.3 mmol/L).
Specimens above this limit must be diluted 1:1 with saline and reassayed.
Multiply the result by 2 to compensate for the dilution.

Calculation:

Triglycerides results are expressed as mg/dl or mmol/L.

$$\text{Triglycerides} = \frac{\text{Abs Unk} \times \text{Conc.Std}}{\text{Abs Std}}$$

Example:

Abs Unk = 0.243

Abs Std = 0.310

Conc. Std = 200 mg/d

$$\text{Triglycerides} = \frac{0.243 \times 200 \text{ mg/dl}}{0.310}$$

Triglycerides = 157 mg/dl

Note: To convert the results into SI units (mmol/L), multiply the result (mg/dl) by 0.0113

Expected Values:

44-148 mg/dl (0.50-1.67 mmol/L)[9]

Due to a wide range of conditions (dietary, geographical, age, etc.) believed to affect normal ranges, it is recommended that each laboratory establish its own reference range.

Performance

1. Assay range: 0-1000mg/dl (0-11.3 mmol/L). Samples that exceed 1000 mg/dl should be diluted with an equal volume of saline and re-assayed. Multiply the result by two.
2. Comparison: A comparison was made between this method and a similar GPO method using 167 samples ranging from 41 mg/dl to 1026 mg/dl. The correlation coefficient was 0.999. Linear regression analysis gave the following equation: This method y = 0.97 x – 4.5 Sy.x = 5.84.
3. Precision: Precision studies were performed following a modification of the guidelines which are contained in NCCLS document EP5-T2.[10]
4. Sensitivity: The sensitivity for this product was investigated by reading the change in absorbance at 500nm for a saline sample, and serum samples with known concentrations. Ten replicates were performed. The results of this investigation indicated that, on the analyzer used, this product showed little or no drift on a zero sample. Under the reaction conditions described, 1mg/dl of triglycerides gives an absorbance of 0.001.

Reference:

1. Fossati, P., Lorenzo, P., Clin. Chem. 28:2077 (1982).

APPENDIX – VII

High Density Lipoprotein (Enzyme Selective Protection Method)

Cholesterol is a component of cell membranes and a precursor for steroid hormones and bile acids synthesized by body cells and absorbed with food. Cholesterol is transported in plasma via lipoproteins, namely complexes between lipids and apolipoproteins. There are four classes of lipoproteins: high density lipoproteins (HDL), low density lipoproteins (LDL), very low-density lipoproteins (VLDL) and chylomicrons. While LDL is involved in the cholesterol transport to the peripheral cells, HDL is responsible for the cholesterol uptake from the cells. The four different lipoprotein classes show distinct relationship to coronary atherosclerosis. LDL-cholesterol contributes to atherosclerotic plaque formation within the arterial intima and is strongly associated with coronary heart disease (CHD) and related mortality. Even with total cholesterol within the normal range an increased concentration of LDL-cholesterol indicates high risk. HDL-cholesterol has a protective effect impeding plaque formation and shows an inverse relationship to CHD prevalence. In fact, low HDL-cholesterol values constitute an independent risk factor. The determination of the individual total cholesterol (TC) level is used for screening purposes while for a better risk assessment it is necessary to measure additionally HDL-cholesterol and LDL cholesterol.

Method

Previous HDL-cholesterol determinations were performed by time consuming precipitation methods HDL-C Immuno FS is a homogeneous method for HDL-cholesterol measurement without centrifugation steps. Antibodies against human lipoproteins are used to form antigen-antibody complexes with LDL, VLDL and chylomicrons in a way that only HDL-cholesterol is selectively determined by an enzymatic cholesterol measurement.

Principle:

LDL, VLDL, Chylomicrons $\xrightarrow{\text{Anti-human β-lipoprotein antibodies}}$ Antigen-antibody complexes + HDL

HDL-cholesterol + H_2O + O_2 $\xrightarrow{\text{CHE \& CHO}}$ Cholest-4-en-3-one + fatty acid + H_2O_2

H_2O_2 + F-DAOS + 4-Aminoantipyrine $\xrightarrow{\text{POD}}$ Blue complex + H_2O

Calculation:

With calibrator

$$\text{HDL-C(mg/dL)} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Calibrator}}$$

Conversion factor:

HDL-C [mg/dL] x 0.02586= HDL-C [mmol/L]

Method Comparison:

A comparison of DiaSys HDL-C Immuno FS (y) with a commercially available test (x) using 100 samples gave following results:

Y = 1.05 x + 0.571 mg/dL; r = 0.995

Reference Range:

- National Cholesterol Education Program (NCEP) guidelines:
- Low HDL-cholesterol (major risk factor for CHD): < 40 mg/dL (< 1.04 mmol/L)
- High HDL-cholesterol ("negative" risk factor for CHD): ≥ 60 mg/dL (≥ 1.55 mmol/L)
- A number of factors contribute to low HDL-cholesterol levels: e.g. overweight and obesity, smoking, physical inactivity, drugs such as beta-blockers and pro gestational agents, genetic factors. Each laboratory should check if reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Reference:

1. Rifai N, Bachorik PS, Albers JJ. Lipids, lipoproteins and apolipoproteins. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999. p. 809-61.

APPENDIX – VIII

Low Density Lipoprotein (Homogeneous Enzymatic Colorimetric Assay)

Principle:

Direct determination of serum LDLc (low-density lipoprotein cholesterol) levels without the need for any pre-treatment or centrifugation steps. The assay takes place in two steps

1. **Elimination of lipoprotein no –LDL:**

   Cholesterol esters CHE cholesterol+ fatty acids

   Cholesterol $+O_2$ CHOD 4-Cholestenone + H_2O_2

   $2H_2O_2$ catalase $2H_2O + O_2$

2. **Measurement of LDLc:**

   Cholesterol esters CHE cholesterol+ fatty acids

   Cholesterol$+O_2$ CHOD 4-Cholestenone $+H_2O_2$

   $2H_2O_2$ +TOOS+4-AA POD $4H_2O$ + Quinonimine

   The intensity of the color formed is proportional to the LDLc concentration in the sample.

Procedure:

1. Assay conditions

   Wavelength……600 ±10 nm

   cuvette……1cm. light path

   Temperature……37°C

2. Adjust the instrument to zero with distelled water.

3. Pipette into a cuvette

	Blank	**Standard**	**Sample**
R1(µL)	**300**	**300**	**300**
Standard(µL)	**-**	**4**	**-**
Sample(µL)	**-**	**-**	**4**

4 Mix and incubate for 5 minutes at 37°C.

5. Add:

R2**(µL)**	100	100	100

6. Mix and incubate for 5 minutes at 37°C.

7. Read the absorbance (A) , against the blank.

Calculations:

$$\frac{\text{(A)Sample}}{\text{(B) Sample}} \times \text{Standard Concentration}$$

=mg/dL of LDLc in the sample

Conversion factor: mg/dL x 0.02586= mmol/L

Reference values:

RISK	LDL-Cholesterol level
Optimal	< 100 mg/dl (2.59 mmol/l)
Near optimal	100-129 mg/dl (2.59-3.34 mmol/l)
Borderline	130-159mg/dl (3.37-4.12mmol/l)
High	160-189mg/dl (4.14-4.89 mmol/l)
Very high	≥ 190mg/dl (4.92 mmol/l)

Interferences:

No interferences were observed with ascorbic acid up to 50 mg/dl, hemoglobin up to 500 mg/dl, bilirubin up to 30 mg/dl, rheumatoid factors up to 1000 IU/ml of lipaemic samples up to 1200 mg/dl. Lipaemic samples with a triglyceride concentration > 1200 mg/dl should be diluted 1/10 with NaCl 9 g/L and multiply the result by 10

Reference:

Nauck, M., Warnick, G. R., & Rifai, N. (2002). Methods for measurement of LDL-cholesterol: A critical assessment of direct measurement by homogeneous assays versus calculation. *Clinical Chemistry*, *48*(2), 236–254. https://doi.org/10.1093/clinchem/48.2.236

APPENDIX - IX

Estimation of Glucose (GOD/PAP)

Glucose is the reducing monosaccharide that serves as the principal source of cellular energy in the body. It enters into the cell under the influence of insulin and undergoes a series of chemical reactions to produce energy. Lack of insulin or resistance to its action at the cellular level causes diabetes. Therefore, in diabetes mellitus the blood glucose level are very high.

Principle:

Glucose oxidase (GOD) converts the sample Glucose into gluconate. The Hydrogenperoxide (H2O2) produced in the reaction is degraded by peroxidase (POD) and gives a colored product Phenol and 4-Aminoantipyrine which is measurable using Trinder indicator reaction at 505 nm. The increase in absorbance correlates with the glucose concentration of the sample.

Glucose+O2 $\xrightarrow{\text{GOD}}$ Gluconic acid+H_2O_2

$2H_2O_2$+Phenol+4-Aminoantipyrine $\xrightarrow{\text{POD}}$ Red quinone+$4H_2O$

Reagents 1.

1)Reagent (R1)

Phosphatase buffer, pH:7.40	100 mmol/l
Phenol	10 mmol/l
4-Aminoantipyrine	0.3 mmol/l
Glucose oxidase	10000 U/l
Peroxidase	700 U/l

2) Glucose standard

Ready for use. For details please check the insert.

Available only in Cat. No.: 46861S and 46862S

Procedure:

Preparation and stability of working reagent

The reagent is ready for use.

If the absorbance of working reagent is higher than 0.1 at 492 nm the reagent can not be used.

Assay conditions

Wavelength	505(492-520) nm
Temperature	37C
Cuvette	1cm light path
Method	Endpoint(increasing)
Read against	Reagent blank

Pipette into cuvette

	Blank	**Standard**	**Sample**
Working reagent	1ml	1ml	1ml
Distilled water	10µl		
Standard		10µl	
Sample			10µl

Mix and measure the absorbance (A) after a five-minute incubation.

Calculation:

$$\frac{A_{Sample}}{A_{Sample}} \times C_{standard} = C_{Sample}$$

A= Absorbance C=Concentration

Quality control:

A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measure to be taken if values fall outside the limits.

Note:

1) With this assay the determination of glucose concentration in urine is not acceptable, because ascorbic acid influences the measurement.
2) The reference method of glucose determination is the hexokinase and the glucose-6-phosphate-dehydrogenase (HK/G-6- PDH) UV test (It is also suitable for the determination of glucose concentration in urine).
3) Do not use reagents after the expiry date stated on each reagent container label.
4) Do not use products, test solutions and reagents described above for any purpose other than described here in.

Reference:

Trinder, P. (1969): Determination of glucose in blood using glucose oxidase with an alternative oxygen ac- ceptor. Annals of Clinical Biochemistry, 6, 24-27

APPENDIX – X

Fasting Insulin (Enzyme- Linked Immunosorbent Assay)

Principle:

The essential reagents required for an immuneenzymometric assay include high affinity and specificity antibodies (Ab), (enzyme conjugated and immobilized), with different and distinct epitope recognition, in excess, and native antigen (Ag). In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal insulin antibody. Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation.

$^{Enz}Ab_{(M)} + Ag_{Ins} + {}^{Btn}Ab_{(M)} \rightleftharpoons {}^{Enz}\mathbf{Ab_{(M)} - Ag_{Ins} - {}^{Btn}Ab_{(M)}}$

$^{Btn}Ab_{(M)}$ = Biotinylated Monoclonal Ab (Excess Quantity)

Ag_{Ins} = Native Antigen (Variable Quantity)

$^{Enz}Ag_{(M)}$ = Enzyme labeled Monoclonal Ab (Excess Quantity)

$^{Enz}Ab_{(M)} - Ag_{Ins} - {}^{Btn}Ab_{(M)}$ = Antigen-Antibody Complex

ka = Rate Constant of Association

k-a = Rate Constant of Dissociation

K = ka / k-a = Equilibrium Constant

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:

$^{Enz}Ab_{(M)} - Ab_{Ins} - {}^{Btn}Ab_{(M)} + Streptavidin_{C.W}$ = Immobilize complex

StreptavidinC.W. = Streptavidin immobilized on well

Immobilized complex = sandwich complex bound to the solid surface

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

Materials Provided

A. Insulin Calibrators – 2.0 ml/vial (Dried) Six (6) vials of references for Insulin antigen at levels of 0**(A),** 5 **(B),** 25**(C),** 50**(D),** 100**(E)** and 300**(F)** µIU/ml. Reconstitute each vial

with 2ml of distilled or deionized water. The reconstituted calibrators are stable for sixty (60) days 2-8°C. A preservative has been added.

Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 1st IRP 66/304.

B. Insulin Enzyme Reagent —13ml/vial

One (1) vial containing enzyme labeled affinity purified monoclonal mouse x-insulin IgG, biotinylated monoclonal mouse x-insulin IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Streptavidin Coated Plate -- 96 wells

One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate – 20 ml

One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2- 30°C.

E. Substrate A –7.0ml/vial

One (1) bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

F. Substrate B –7.0ml/vial

One (1) bottle containing hydrogen peroxide (H2O2) in buffer. Store at 2-8°C.

G. Stop Solution – 8.0ml/vial

One (1) bottle containing a strong acid (1N HCI). Store at 2-30°C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.

Note 2: Avoid extended exposure to heat and light. Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.

Note 3: Above reagents are for a single 96-well microplate.

Procedure:

Specimen Collection and Preparation

The specimens shall be blood: serum or plasma in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants (for serum) or evacuated tube(s) containing EDTA or heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the samples(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml of the specimen is required.

Calculation of Result

A dose response curve is used to ascertain the concentration of Insulin in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in **Example 1.**
2. Plot the absorbance for each duplicate serum reference versus the corresponding Insulin concentration in µIU/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of Insulin for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in µIU/ml) from the horizontal axis of the graph (the duplicate of the unknown may be averaged as indicated). In the following example, the average absorbance (0.624) intersects the dose response curve at 66.8 µIU/ml Insulin concentration.

Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimens(s) should not be used.
4. If more than once (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.

9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from DAI IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.

Reference:

Eastham RD, Biochemical Values in Clinical Medicine. 7th Ed Bristol, England, John Wright & Sons, Ltd (1985).

APPENDIX – XI

Visceral Adiposity Index Calculation

$$\text{VAI for Women} = \frac{WC}{36.58 + (1.89 \times BMI)} \times \frac{TG}{0.81} \times \frac{1.52}{HDL}$$

WC - waist circumference (cm); TG - triglycerides (mmol); HDL - high-density lipoprotein (mmol); BMI - body mass index

Variables	**Measurements**	**Conversion Factor^**	
WC	85 cm		
BMI	26.67		
TG	69 mg/dL	TG/ 88.47	0.78 mmol
HDL	38 mg/dL	HDL/ 38.67	0.98 mmol

^Lipid Conversion Factors (Rugge et al., 2011)

$$VAI = \frac{85}{36.58 + (1.89 \times 26.67)} \times \frac{0.78}{0.81} \times \frac{1.52}{0.98}$$

$$= \frac{85}{36.58 + 50.41} \times 0.96 \times 1.55$$

$$= 0.98 \times 0.96 \times 1.55$$

= 1.46

APPENDIX – XII

Lipid Accumulation Product Calculation

LAP for women = (WC - 58) x TG

WC - waist circumference (cm); TG - triglycerides (mmol).

Variables	**Measurements**	**Conversion Factor^**	
WC	85 cm		
TG	69 mg/dL	TG/ 88.47	0.78 mmol

^Lipid Conversion Factors (Rugge et al., 2011)

LAP = (85 – 58) x 0.78

= 27 x 0.78

= 21.06

APPENDIX – XIII

Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) Calculation

$$\textbf{HOMA-IR} = \frac{\textbf{fasting glucose X fasting insulin}}{\textbf{405}}$$

Fasting blood sugar (FBS) in mg/dL; Fasting Insulin (FI) in µIU/ml

Variables	**Measurements**
FBS	103 mg/dL
FI	5.9 µIU/ml

$$\textbf{HOMA-IR} = \frac{103 \times 5.9}{405}$$

$$= \frac{607.7}{405}$$

= 1.5

APPENDIX – XIV

Assessment of Dietary Pattern

Dietary Habits

1. Dietary habit

   ☐ Vegetarian ☐ Non – vegetarian ☐ Ova – vegetarian

2. Frequency of meal consumption

   ☐ 3 times ☐ 3 – 4 times ☐ 4 – 5 times ☐ 6 – 7 times

3. Do you have breakfast daily? ☐ Yes ☐ No

4. If No, how many times do you skip breakfast per week?

   ☐ 1 – 2 ☐ 2-4 ☐ 4 – 5 ☐ 6 – 7

5. Do you perform fasting?

   ☐ Yes ☐ No

6. Is there any meal restriction in their diet?

   ☐ Yes ☐ No

7. Oil used for their cooking?

   _____

8. Are you allergic to any foods? Mention

   ☐ Yes ☐ No _____

9. Do you prefer online ordered foods?

   ☐ Yes ☐ No

Dietary Intake: 3 days dietary recall

Meal Time	**Quantity** (g/ml/cup/ nos)	**Day 1 (Saturday)**	**Quantity** (g/ml/cup/ nos)	**Day 2 (Sunday)**	**Quantity** (g/ml/cup/ nos)	**Day 3 (Monday)**
Early morning						
Breakfast						
Mid-morning						
Lunch						
Tea and snack						
Dinner						
Bed time						

APPENDIX – XV

International Physical Activity Questionnaire – Short Form (IPAQ-SF) 2004

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

_____ days per week

☐ No vigorous physical activities → ***Skip to question 3***

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

_____ hours per day

_____ **minutes per day**

☐ Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

_____ days per week

☐ No moderate physical activities → ***Skip to question 5***

1. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ hours per day

_____ **minutes per day**

☐ Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

2. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ days per week

☐ No walking → ***Skip to question 7***

3. How much time did you usually spend **walking** on one of those days?

_____ hours per day

_____ **minutes per day**

☐ Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

4. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____ hours per day

_____ **minutes per day**

☐ Don't know/Not sur

APPENDIX – XVI

INTENSITY LEVELS OF PHYSICAL ACTIVITY

Categorical score Regular participation is a key concept included in current public health guidelines for physical activity (Pate et al., 1995). Therefore, both the total volume and the number of day/sessions are included in the IPAQ analysis algorithms. There are three levels of physical activity suggested for classifying

populations; these are the new proposed levels, which take account of the concept of total physical activity of all domains. The proposed levels are:

[i] inactive

[ii] minimally active

[iii] HEPA active (health enhancing physical activity; a high active category).

The criteria for these three levels are shown below.

1. Inactive (CATEGORY 1)

This is the lowest level of physical activity. Those individuals who not meet criteria for Categories 2 or 3 are considered insufficiently active' [CATEGORY 1]

2. Minimally Active (CATEGORY 2)

The minimum pattern of activity to be classified as 'sufficiently active' is any one of the following 3 criteria:

a) 3 or more days of vigorous activity of at least 20 minutes per day OR

b) 5 or more days of moderate-intensity activity or walking of at least 30 minutes per day OR

c) 5 or more days of any combination of walking, moderate-intensity or vigorous intensity activities achieving a minimum of at least 600 MET-min/week.

Individuals meeting at least one of the above criteria would be defined as achieving the minimum recommended to be considered 'minimally active' [CATEGORY 2]. This category is more than the minimum level of activity recommended for adults in current public health recommendations, but is not enough for "total PA" when all domains are considered. IPAQ measures total physical activity whereas the recommendations are based on activity (usually leisure-time or recreational) over and above usual daily activities.

3. HEPA active (CATEGORY 3)

A separate category labelled 'HEPA' level, which is a more active category [CATEGORY 3] can be computed for people who exceed the minimum public health physical activity recommendations, and are accumulating enough activity for a healthy lifestyle. This is a useful indicator because it is known that higher levels of participation can provide greater health benefits, although there is no consensus on the exact amount of activity for maximal benefit. Also, in considering lifestyle physical activity, this is a total volume of being active which reflects a healthy lifestyle. It is at least 1.5 - 2 hours of 'being active' throughout the day, which is more than the LTPA-based recommendations of 30 minutes.

In the absence of any established criteria, the IPAQ scientific group proposes this new cutpoint, which equates to approximately at least 1.5 -2 hours of total activity per day, of at least moderateintensity activity. It is desirable to have a 'HEPA' activity category, because in some populations, a large proportion of the population may be classified as 'minimally active' because the IPAQ instrument assess all domains of activity. Category 3 sets a higher threshold of activity and provides a useful mechanism to distinguish variation in sub-population groups.

The two criteria for classification as 'HEPA active' are:

a) vigorous-intensity activity on at least 3 days achieving a minimum of at least 1500 MET-minutes/week OR

b) 7 or more days of any combination of walking, moderate-intensity or vigorous intensity activities achieving a minimum of at least 3000 MET-minutes/week.

Continuous score

Data collected with IPAQ can be reported as a continuous measure and reported as median METminutes. Median values can be computed for walking (W), moderate-intensity activities (M), and vigorous-intensity activities (V) using the following formulas:

MET values and Formula for computation of Met-minutes

Walking MET-minutes/week = 3.3 * walking minutes * walking 'days'

Moderate MET-minutes/week = 4.0 * moderate-intensity activity minutes * moderate days

Vigorous MET-minutes/week = 8.0 * vigorous-intensity activity minutes * vigorous-intensity days

A combined total physical activity MET-min/week can be computed as the sum of Walking + Moderate + Vigorous MET-min/week scores.

IPAQ Sitting Question

The IPAQ sitting question is an additional indicator variable and is not included as part of any summary score of physical activity. Data on sitting should be reported as median values and interquartile range. To-date there are few data on sedentary (sitting) behaviors and no well-accepted thresholds for data presented as categorical levels.

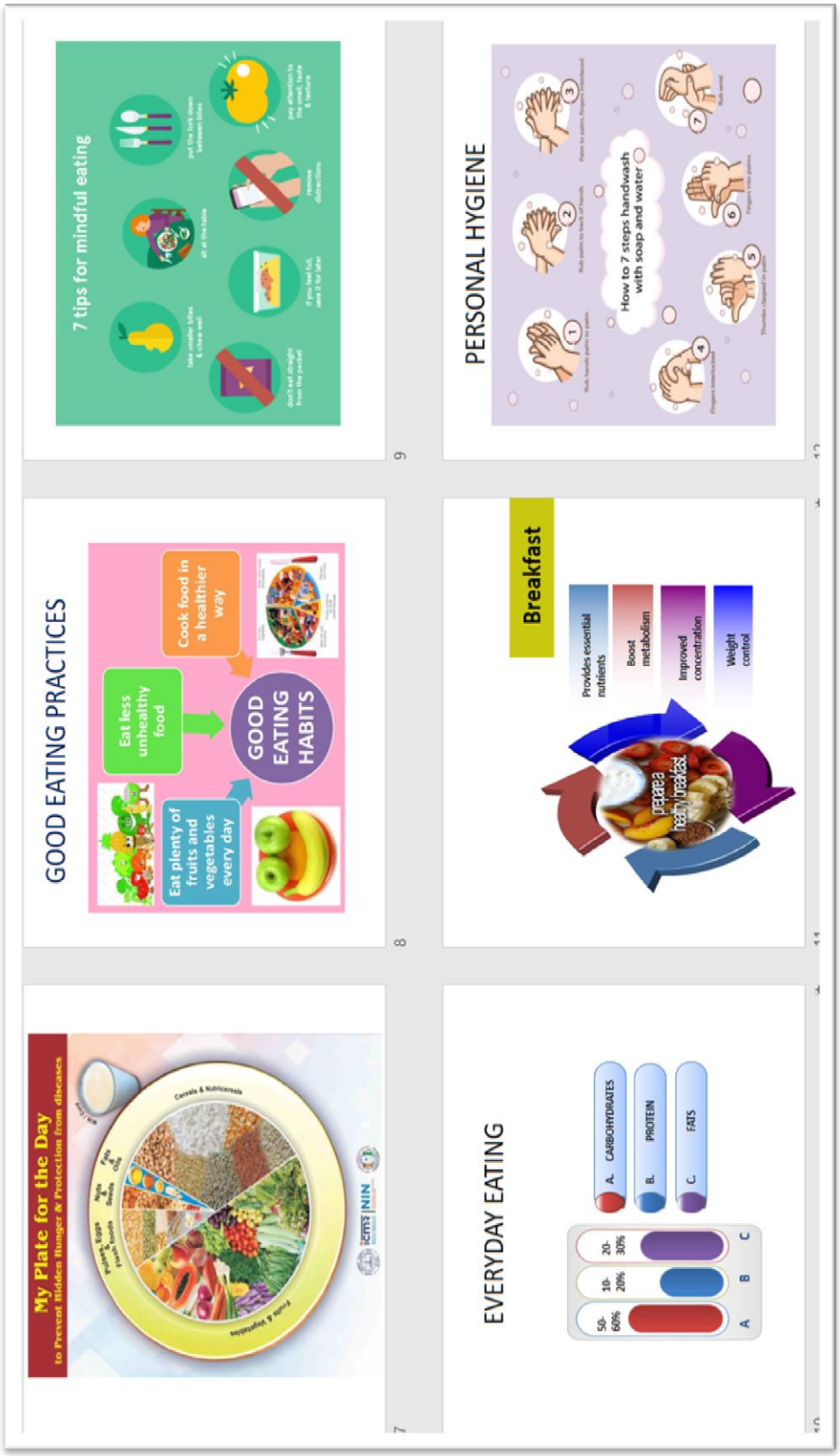
APPENDIX – XVII

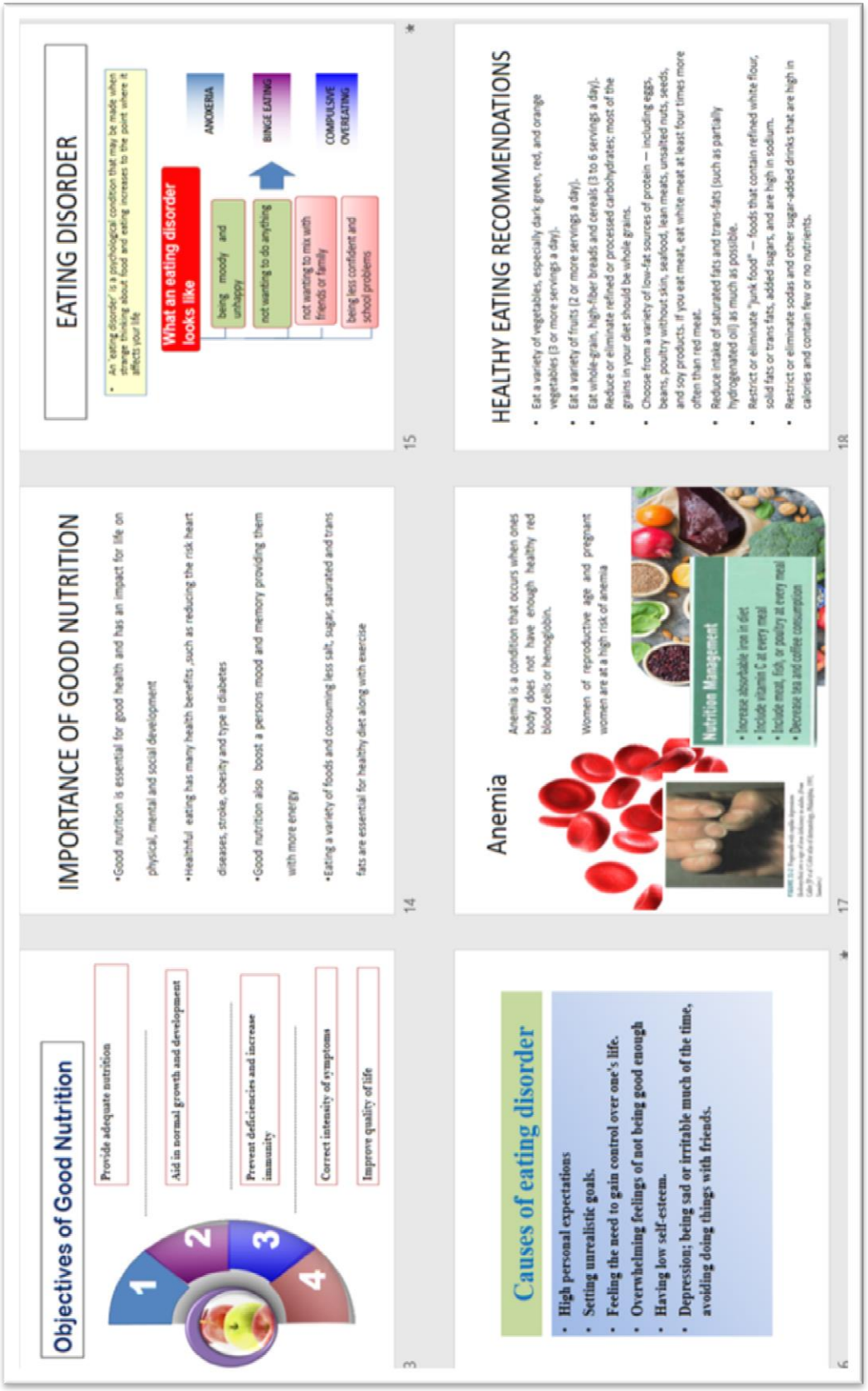
ANTHROPOGENS

Determinants	Decrease risk	Increase risk	Moderators
Nutrition/ Diet	Fruit/vegetables Dietary fibre Whole grains Food variety Seafood Healthy eating patterns	High total energy High energy density Excess processed foods High GI foods Sat./trans fats Sugars Salt Excessive Alcohol Sugared soft drinks Processed/red meat	Binge eating/drinking Social/holiday eating "Restrained" eating Feasting Culture Habits
(In) Activity	Aerobic exercise Resistance exercise Stretching Stability Leisure Activity Incidental activity	Sitting/sedentary work Overexercise	Fear of crime Fatigue/laziness Discomfort/injury/ Early experiences Energy-saving devices Obesity Habits
Inadequate sleep	REM sleep Bed-time Hypersomnia Nutrition Exercise/fitness	Stress Entertainment Sleep Disorders Overheating Interactive Media Alcohol/drugs	Activity before sleep Stress Anxiety/depression Obesity habits
Smoking/ alcohol	Appropriate medication	Recreational drugs Cigarette smoking Alcohol use Iatrogenesis	Stress, anxiety, and depression Peer/social pressure Addiction Binge drinking Habit
Over and under-exposure	Sunlight light stimulation	Sunlight (excess) Sunlight (inadequate) Low humidity/ asbestos Radiation	Peer/social pressure Cultural influences Habit

APPENDIX XVIII

POWER POINT PRESENTATION


My Plate for the Day
to Prevent Hidden Hunger & Protection from diseases
Cereals & Nutricereals
Fruits & Vegetables
icmr | NIN
GOOD EATING PRACTICES
Eat less unhealthy food
Cook food in a healthier way
GOOD EATING HABITS
Eat plenty of fruits and vegetables every day
Breakfast
Provides essential nutrients
Boost metabolism
Improved concentration
Weight control
prepare a healthy breakfast
EVERYDAY EATING
A. CARBOHYDRATES
B. PROTEIN
C. FATS
50-60%
10-20%
20-30%
A B C
7 tips for mindful eating
PERSONAL HYGIENE
How to 7 steps handwash with soap and water


EATING DISORDER
An 'eating disorder' is a psychological condition that may be made when strange thinking about food and eating increases to the point where it affects your life
What an eating disorder looks like
being moody and unhappy
not wanting to do anything
not wanting to mix with friends or family
being less confident and school problems
ANOREXIA
BINGE EATING
COMPULSIVE OVEREATING
15
HEALTHY EATING RECOMMENDATIONS
Eat a variety of vegetables, especially dark green, red, and orange vegetables (3 or more servings a day).
Eat a variety of fruits (2 or more servings a day).
Eat whole-grain, high-fiber breads and cereals (3 to 6 servings a day). Reduce or eliminate refined or processed carbohydrates; most of the grains in your diet should be whole grains.
Choose from a variety of low-fat sources of protein — including eggs, beans, poultry without skin, seafood, lean meats, unsalted nuts, seeds, and soy products. If you eat meat, eat white meat at least four times more often than red meat.
Reduce intake of saturated fats and trans-fats (such as partially hydrogenated oils) as much as possible.
Restrict or eliminate "junk food" — foods that contain refined white flour, solid fats or trans fats, added sugars, and are high in sodium.
Restrict or eliminate sodas and other sugar-added drinks that are high in calories and contain few or no nutrients.
18
IMPORTANCE OF GOOD NUTRITION
•Good nutrition is essential for good health and has an impact for life on physical, mental and social development
•Healthful eating has many health benefits ,such as reducing the risk heart diseases, stroke, obesity and type II diabetes
•Good nutrition also boost a persons mood and memory providing them with more energy
•Eating a variety of foods and consuming less salt, sugar, saturated and trans fats are essential for healthy diet along with exercise
14
Anemia
Anemia is a condition that occurs when ones body does not have enough healthy red blood cells or hemoglobin.
Women of reproductive age and pregnant women are at a high risk of anemia
Nutrition Management
Increase absorbable iron in diet
Include vitamin C at every meal
Include meat, fish, or poultry at every meal
Decrease tea and coffee consumption
17
Objectives of Good Nutrition
Provide adequate nutrition
Aid in normal growth and development
Prevent deficiencies and increase immunity
Correct intensity of symptoms
Improve quality of life
1
2
3
4
3
Causes of eating disorder
High personal expectations
Setting unrealistic goals.
Feeling the need to gain control over one's life.
Overwhelming feelings of not being good enough
Having low self-esteem.
Depression; being sad or irritable much of the time, avoiding doing things with friends.
6

EXERCISE

World Health Organization

Majority of adolescents worldwide are not sufficiently physically active, putting their current and future health at risk

(WHO, 2019)

- Up to **5 million deaths a year could be averted** if the global population was more active (WHO, 2020)
- More than **80% of the world's adolescent population** is insufficiently physically active (WHO, 2020)
- Health benefits - **cardiorespiratory and muscular fitness, bone and cardio metabolic health, and positive effects on weight.**

PHYSICAL ACTIVTY RECOMMENDATION

- Maintain or work toward a healthy weight.
- Be physically active every day
- Get moderate to vigorous physical activity for at least 30 minutes a day 5 days a week.
- Healthy eating provides the sustained energy you need to be physically active.
- Learn to manage your stress with exercise, healthy eating, relaxation, and good coping skills

APPENDIX - XIX

DIET INTERVENTION

Measurements

1 teaspoon (down) = 5 grams

1 tablespoon (up) = 15 grams

Measurements in cooked weight

1 katori = 150 ml or grams

½ katori = 75 ml or grams

Medium size tea cup = 200 ml

1 cup/ glass = 250 ml or grams

1 Katori

Katori for sambar, chutney and salad

200 kcals Diet Plan (Vegetarian)

Meal time	Menu	Quantity
Early morning 6.00 – 6.30 am	Coffee (add milk after removing the fat layer on the top of milk) Jaggery	1 glass 1 teaspoon
Breakfast 8.30-9.00 am	**Option I** Idly/ Dosa/ Chapathi Sambar **or** Mint chutney or any other chutney except coconut chutney Any fruit (Papaya/ Guava/ Watermelon)	 2 1/2 katori 1/2 slices 5 small pieces
Mid-morning 11-11.30 am	Vegetable Soup/ Amla and cucumber smoothie	½ Katori 1 glass
Lunch 12.30-1.30 pm	**Option I** Vendaya keerai Chapathi Dhal/ any vegetable gravy for chapathi Carrot & Cucumber raita **or** **Option II** Rice/ variety rice Drumstick leaves sambar/ for rice Beans or any vegetable porriyal Butter milk with hing Carrot & Cucumber Salad	 2 (medium size) ½ katori ½ katori ½ cup ½ katori ½ katori 1 medium glass Each 3 slices
Evening 4.30-5.30 pm	Tea (add milk after removing the fat layer on the top of milk) Jaggery Puffed rice with onion and carrot	1 glass 1 teaspoon ½ katori
Dinner 8.00-9.00 pm	Broken wheat upma/ Avul upma/ Sevai Tomato Chutney Any fruit (Pineapple **or** Apple)	1 cup ½ katori 2 slices/ 3 slices

200 kcals Diet Plan (Non - Vegetarian)

Meal time	Menu	Quantity
Early morning 6.00 – 6.30 am	Coffee (add milk after removing the fat layer on the top of milk) Jaggery	1 glass 1 teaspoon
Breakfast 8.30-9.00 am	**Option I** Egg Dosa Onion tomato chutney **or** **Option II** Wheat puttu Kadala curry Egg	 1 1/2 katori 1 cup ½ Katori 1
Mid-morning 11-11.30 am	Sprouts salad **or** Guava	½ Katori 1 medium size
Lunch 12.30-1.30 pm	**Option I** Chapathi Chicken gravy for chapathi Chicken pieces Tomato & Cucumber Salad Butter milk **or** **Option II** Rice Chicken/Fish kolambu Chicken/ Fish (small) Butter milk with hing Carrot & Cucumber Salad	 2 (medium size) ½ katori 3 pieces Each 3 slices 1 medium glass ½ cup ½ katori 3 pieces (big fish means 1) 1 medium glass Each 3 slices
Evening 4.30-5.30 pm	Puffed rice with onion & carrot Tulasi Tea (without sugar)	½ katori 1 medium glass
Dinner 8.00-9.00 pm	**Option I** Pumpkin soup Egg Apple **or** **Option II** Mini Dosa/ Idly Brinjal chutney Egg Pomegranate	 1 cup 1 3 slices 6-8 pieces ½ katori 1 ½ katori

300 kcals Diet Plan (Vegetarian)

Meal time	Menu	Quantity
Early morning 6.00 – 6.30 am	Coffee (add milk after removing the fat layer on the top of milk) Jaggery	1 glass 1 teaspoon
Breakfast 8.30-9.00 am	**Option I** Idly/ Oats Upma/ Broken Wheat Upma Sambar **or** Mint chutney or any other chutney except coconut chutney Any fruit (Papaya/ Guava/ Watermelon)	 2 1/2 katori 1/2 katori 1/2 slices 5 small pieces
Mid-morning 11-11.30 am	Vegetable Soup/ Carrot and cucumber salad	½ Katori
Lunch 12.30-1.30 pm	**Option I** Chapathi Palak gravy Lady's finger Onion raita **or** **Option II** Rice/ Vegetable Rice Ash gourd sambar/ for rice Beans or any vegetable porriyal Butter milk with hing Carrot & Cucumber Salad	 2 (medium size) ½ katori ½ katori ½ katori ½ cup ½ katori ½ katori 1 medium glass Each 3 slices
Evening 4.30-5.30 pm	Hibiscus Tea Jaggery Puffed rice with onion and carrot	1 glass 1 teaspoon ½ katori
Dinner 8.00-9.00 pm	Avul upma/ Sevai Tomato Chutney Any fruit (Pineapple **or** Apple)	1 cup ½ katori 2 slices/ 3 slices

300 kcals Diet Plan (Non - Vegetarian)

Meal time	Menu	Quantity
Early morning 6.00 – 6.30 am	Coffee (add milk after removing the fat layer on the top of milk) Jaggery	1 glass 1 teaspoon
Breakfast 8.30-9.00 am	**Option I** Ragi Dosa Onion tomato chutney Egg **or** **Option II** Wheat puttu Kadala curry	 1 1/2 katori 1 1 cup ½ Katori
Mid-morning 11-11.30 am	Sprouts salad **or** Guava	½ Katori 1 medium size
Lunch 12.30-1.30 pm	**Option I** Chapathi Chicken gravy Chicken pieces Tomato & Cucumber Salad Butter milk **or** **Option II** Rice Chicken/Fish kolambu Chicken/ Fish (small) Butter milk with hing Carrot & Cucumber Salad	 2 (medium size) ½ katori 2 medium pieces Each 3 slices 1 medium glass ½ cup ½ katori 3 pieces (big fish means 1) 1 medium glass Each 3 slices
Evening 4.30-5.30 pm	Puffed rice with onion & carrot Tulasi Tea (without sugar)	½ katori 1 medium glass
Dinner 8.00-9.00 pm	**Option I** Pumpkin soup Egg (white) Apple **or** **Option II** Carrot and onion uthappam/ Rava Idly Brinjal and ridge gourd chutney Pomegranate	 1 cup 1 3 slices 1 2 ½ katori ½ katori

500 kcals Diet Plan (Vegetarian)

Meal time	**Menu**	**Quantity**
Early morning 6.00 – 6.30 am	Coffee (add milk after removing the fat layer on the top of milk) Jaggery	1 glass 1 teaspoon
Breakfast 8.30-9.00 am	**Option I** Bread salsa sandwich/ Wheat Dosa Coriander chutney	 2 ½ katori
Mid-morning 11-11.30 am	Cucumber salad **or** Orange/Guava	½ katori 1 medium
Lunch 12.30-1.30 pm	**Option I** Rice Kadala kolambu Butter milk Capsicum stir fry **or** **Option II** Rice Green gram masiyal Ridge gourd poriyal Butter milk with hing	 ½ katori ½ katori 1 medium glass ½ katori ½ cup ½ katori ½ katori 1
Evening 4.30-5.30 pm	Chana chat **or** Puffed rice Tea	½ katori 1 medium glass
Dinner 8.00-9.00 pm	**Option I** Adai Dosa/ Sevai/ Phulka Tomato chutney/ Channa masala Any fruit (Papaya/ Grapes)	 2/ 1 katori ½ katori 1 long slice/ 5-8 grapes

500 kcals Diet Plan (Non- Vegetarian)

Meal time	Menu	Quantity
Early morning 6.00 – 6.30 am	Coffee (add milk after removing the fat layer on the top of milk) Jaggery	1 glass 1 teaspoon
Breakfast 8.30-9.00 am	**Option I** Bread and Omelette/ Ragi Dosa Mint chutney	 2 ½ katori
Mid-morning 11-11.30 am	Cucumber salad **or** Green gram sundal	½ katori ½ katori
Lunch 12.30-1.30 pm	**Option I** Rice Fish kolambu Lemon Rasam **or** **Option II** Chapathi Chicken Gravy Onion and Tomato salad	 ½ katori ½ katori with 1 piece 1 medium glass ½ cup ½ katori with 2 medium pieces ½ katori
Evening 4.30-5.30 pm	Chana chat **or** Puffed rice chat Tea	½ katori 1 medium glass
Dinner 8.00-9.00 pm	**Option I** Idly/ Sevai/ Phulka Tomato chutney/ Channa masala Any fruit (Papaya/ Grapes)	 2/ 1 katori ½ katori 1 long slice/ 5-8 grapes

FOODS INCLUDED in Moderation	FOODS INCLUDED in liberally	FOODS EXCLUDED
Rice, millet, wheat	Other vegetables	Processed foods
Carrot & Beetroot	Green leafy vegetables	Junk foods
Milk	Buttermilk,	Fried foods, ghee, vanaspathi
Nuts	Clear soups	Soft drinks
Mango	Low carbohydrate fruits and vegetables	Sweets, chocolates, jam, jellies, ice cream
Potato	Sprouts	Fatty meat cuts, fatty fish

Sample Meal Plates:

Breakfast

Option I: Vegetarian

Wheat puttu with Kadala kolambu
Omelette

Option II: Non- Vegetar

Bread 2 slices (without butter),
Apple 3 slices

Lunch

Option 1

Rice
Egg curry
Carrot, cucumber and onion salad
Mango 1 slice

Option II

Vendaya keerai Chapathi 2

Fish kolambu with 3 pieces
Bottle gourd poriyal
Carrot and cucumber salad

Dinner

Option 1

Ragi Dosa 2
Brinjal & Tomato curry
Apple 3 slices

Option II

Mini Dosa 6 pieces
Egg 1
Ivy gourd (Kovakai poriyal)

General Guidelines:

1. The timing of the meals is very important and kindly assure to eat at specific time.
2. The pictures shown is a sample and it can be altered
3. Kindly avoid processed foods, bakery foods, junk and fried foods till the end of intervention
4. Try including atleast 1 fruit daily. All the pictures attached has fruits, try to include it either in breakfast or mid-morning or dinner.
5. Follow the foods included and excluded tabular column.
6. Sunday is cheat day. You can eat what is prepared in home but stay away from processed foods, bakery and junk foods

APPENDIX – XX

REAL TIME COUNSELLING

FOOD PLATE ADJUSTMENTS

BEFORE

AFTER

BEFORE

AFTER

BEFORE

AFTER

BEFORE

AFTER

APPENDIX – XXI

PHYSICAL ACTIVITY INTERVENTION

Physical Activity

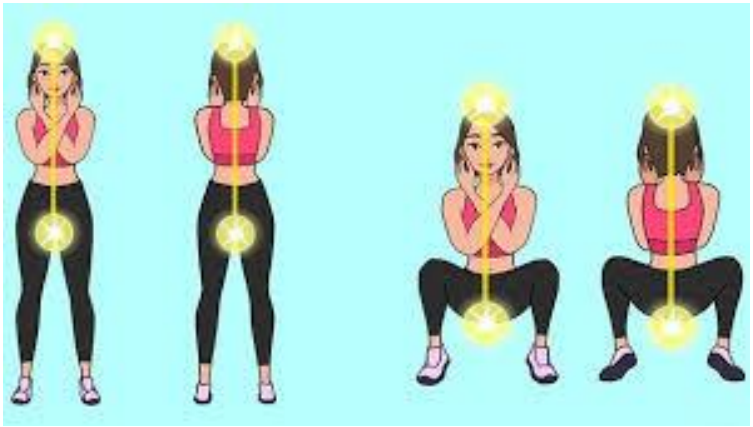
Morning

30 mins Brisk walking (Listen to favourite 6 songs)

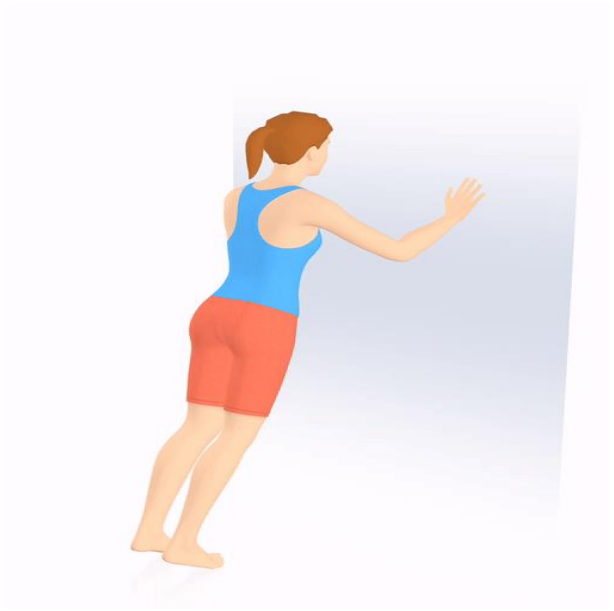
Evening

1. Jumping jacks = 30

2. Thoopukaranam ×10 (2 sets) = 20



3. Wall push ups = 20



4. Side-lying leg lift (left) = 15
5. Side-lying leg lift (right) = 15

6. Abdominal Crunches = 10

7. Bicycle crunches = 20

8. Mountain climbers = 15

9. Toe touch = 10

General Guidelines

- Don’t sit for a long time in a place. At the end of every class, get up stretch your body, go and fetch some water and drink.
- Hydrate well
- The intensity of the exercises will be increased after one or two weeks depending upon your body’s reaction to the exercises

The participants are advised and recommended to follow the physical activity for 5 day per week