E.V.E LABORATORY

Clinical Research Protocol

The Study of Tea flower extract from Buds (*Camellia sinensis)* on Obesity Control (SCAS): A double-blinded randomized control trial of the efficacy for obesity control.

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1 Abstract

Tea flower extracts from buds (*Camellia sinensis*, Theaceae) and have been studied on animal for the prevention of chronic diseases, including obesity. Obesity currently affects >20% of adults in the United States and is a risk factor for chronic diseases such as type II diabetes, cardiovascular disease, and cancer. Given this increasing public health concern, the development use of agents for the prevention of obesity would be of tremendous benefit.

It is reported that the public health impact of obesity in terms medical spending in the United States in 2006 was approximately $119 billion. Although surgical and pharmacological methods have been developed to treat obesity, these treatments can be costly and are not without potential adverse effects. The development of dietary agents for the prevention or treatment of obesity, and their use in combination with exercise and changes in energy consumption, could represent a cost-effective and safe means to deal with this growing public health crisis.

Previous study has shown that the tea flower extracts from buds of *Camellia sinensis* for those activities of inhibition of glucose absorption, inhibition of lipid absorption and gastro-protective effects were recognized. Especially, the extracts from the flowers of the tea tree were found to suppress serum triglyceride elevation and also loosing body weight in lipid-rich diet mice. Based on those facts, the clinical trial is designed and conducted to examine the efficacy of tea flower (*Camellia sinensis)* extracts from buds of on Obesity control in combination with other nutrients.

2 Clinical Trial Information

2.1 Back Ground – Research Material

Tea flowers, the flower buds of *Camellia sinensis*, are used as a food garnish in Japanese style dishes (e.g., batabata-cha in Shimane prefecture) or drinks in Japan(**1**)(e.g., hanaban-cha in Shimane and Kouchi prefectures or botebote-cha in Niigata prefecture.)
Camellia sinensis is the species of plant whose leaves and leaf are used to produce tea. It is of the genus *Camellia*, a genus of flowering plants in the family Theaceae. White tea, green tea, oolong, pu-erh tea and black tea are all harvested from this species, but are processed differently to attain different levels of oxidation.Kukicha

(twig tea) is also harvested from *Camellia sinensis*, but uses twigs and stems rather than leaves. Common names include tea plant, tea tree, and tea shrub.
Chinese *Camellia sinensis* is native to mainland China, South and Southeast Asia, but it is today cultivated across the world in tropical and subtropical regions. It is an evergreen shrub or small tree that is usually trimmed to below two metres (six feet) when cultivated for its leaves. It has a strong taproot. The flowers are yellow-white, 2.5–4 cm in diameter, with 7 to 8 petals.

The leaves are 4–15 cm long and 2–5 cm broad. Fresh leaves contain about 4% caffeine **(2)**. The young, light green leaves are preferably harvested for tea production; they have short white hairs on the underside. Older leaves are deeper green. Different leaf ages produce differing tea qualities, since their chemical compositions are different. Usually, the tip (bud) and the first two to three leaves are harvested for processing. This hand picking is repeated every one to two weeks

2.2 Study Rational – Animal Studies

The flower buds of *Camellia sinensis* (Tea Flower, 'Chaka' in Japanese) have been used as a food garnish in Japanese-style dishes: for example, 'Botebotecha' in Shimane prefecture. The methanolic extract from the flower buds of *Camellia sinensis* cultivated in Fujian Province showed inhibitory effects on body weight gain and the weight of visceral fats in high-fat diet-fed mice and/or Tsumura Suzuki Obese Diabetic (TSOD) mice.
However, the chemical constituentsand pharmacological properties of these flower buds have not yet been clarified. In the studies on the bioactive constituents of medicinal flowers, it is reported the isolation and structural elucidation of acylated polyhydroxyoleanane-type triterpene oligoglycosides, floratheasaponins A–L from the flower buds of C. sinensis cultivated in Japan and in Anhui Province, China, (3-7) and chakasaponins I–III (1–3), V, and VI from the flower buds of C. sinensis cultivated in Fujian Province, China (8). These saponins showed anti-hyperlipidemic, anti-hyperglycemic, anti-allergic, and gastroprotective effects (3-8).

2.3 Study Rational - Clinical Studies

The treatments of obesity are basically control of daily diet and Exercise. However, with the development of the drug acting as inhibitor of lipid metabolise and also lipid adsorption, the Efficacy of anti obesity drug is now in a spot light in Japan, Only the Mazingdol is approved as the ant obesity drug at present. At the US and other nations, Olirstate, as the lipase inhibitor, has been used in clinical treatment. But the following side effects such as pass gas even at early stage of administration,

fatty stools are often recognized (9).
The flower extract from buds Of *Camellia sinensis* has been evaluating for active substances for treatment and prevention of Obesity. It has been founded the physiologically active substance for suppression of the blood sugar level elevation, and inhibition of Triglycerides as well as absorption of gastro-protective activity in the tea tree flower. They confirmed the possibility on the substances for treating obesity.
The Shenyang Pharmaceutical University Hospital, China, Has evaluated the substances through one month clinical trial (9), with double blind, placebo-controlled. Through statistical analysis on the measurement of body weight, waist, hips, and blood indicators, significant difference in body weight loss is confirmed statistically. (100mg/day; average 2.50kgs (96.3%)/month, 300mg/day; average-2.10kgs (96.8%)/month, 1000mg/day; average-1.70kgs (99.2kgs)/month), at the same time, the significant decrease is also observed on the length around shoulder, waist and hips compared with the group treated with placebo.
In conclusion, there is no adverse effect reported in the trail, none of abnormalities were observed in the blood biochemical analysis which clinically represents the confirmation of safety of the treatment with the extracts of Tea tree flower from Buds.
The trail has confirmed and claimed that the treatment of the extracts of tea Tree flower buds results in body weight loss and effect for improvement of lipid metabolism, without any adverse effect on individual's health condition.

2.4 Study Rational - Prevalence of Obesity

The global epidemic of obesity

The prevalence of obesity is increasing in most part of the world, affecting men, women and children. Furthermore, obesity is no longer just a concern for developed countries, but it is becoming an increasing problem in many developing countries **(10)**.

2.4.1 Prevalence of obesity

It should be noted that it is often difficult to make a direct comparison of the prevalence of obesity between countries due to the inconsistent classifications used for obesity. This problem may be overcome with the adoption of the WHO standardised classification for obesity, in future surveys. From available data, the world-wide prevalence of obesity has been found to range from less than 5% in

rural China, Japan and some African countries to levels as high as 75% of the adult population in urban Samoa. (Appendices, Figure 1) provides examples of the varying prevalence of obesity within different countries. Obesity levels also vary depending on ethnic origin. In the USA, particularly among women, there are large differences in the prevalence of obesity between populations of the different ethnic origins within the same country (11).

The growing prevalence of obesity among children is also a major concern (Appendices, Table 3). The lack of agreement in defining obesity in children and adolescents has made it difficult to estimate the true prevalence. The International Obesity Task Force (IOTF) developed a new approach to defining childhood overweight and obesity to make it consistent with the adult definition (12). (http://bmj.com/cgi/content/abridged/320/7244/1240) However, using existing WHO standards, data from 79 developing countries and a number of industrialised countries suggests that about 22 million children under 5 years old are overweight world-wide (WHO 1998). There is also evidence that this problem is increasing; in the USA, the percentage of overweight children (aged 5-14 years) has doubled in the last 30 years, from 15% to 32%(13).

2.4.2 Trends and projections

Many countries have experienced a startling increase in obesity rates over the last 10-20 years (Appendices, Figure 2). Over the past decade levels have increased on average between 10-40% (Seidell & Flegal 1997). In England the prevalence of obesity has doubled since 1980. Based on current trends, it is predicted that the levels of obesity will continue to rise unless action is taken now. The WHO recently stated "the growth in the number of severely overweight adults is expected to be double that of underweight during 1995-2025" (WHO 1998). Crude projections, from extrapolating existing data, suggest that by the year 2025 levels of obesity could be as high as 45-50% in the USA, between 30-40% in Australia, England and Mauritius and over 20% in Brazil (Appendices, Figure 3)(14).

2.4.3 Key patterns associated with obesity

A number of factors have been linked to obesity, including age, gender and socio-economic status. In developed countries the natural pattern with age is an increase in body weight with ageing, at least up to 50-60 years old (in both men and women). The relationship between obesity and age is similar in developing countries, but the maximum rates of obesity tend to be reached at an earlier age (e.g. 40 years old). The decline in prevalence after this peak is thought to be partly attributed to lower

survival rate of obese individuals. Clear gender difference are seen in most countries with more women than men being obese (BMI ³ 30). In contrast, the proportion of men who are overweight (BMI 25.0-29.9) tends to be greater than women (Appendices, Figure 4). Patterns have also emerged across socio-economic groups. In developed countries levels of obesity are higher in the lower socio-economic groups. In developing countries this relationship is reversed. The transition from a rural to an urban lifestyle is associated with increased levels of obesity, which has been linked with dramatic changes in lifestyles (e.g. increased consumption of high energy dense foods and decrease in physical activity). As stated in section 3.1 ethnicity is also thought to be feature associated with the variation in levels of obesity (15).

2.4.4 Health, social and economic costs of obesity

Obesity has a great number of negative health, social and economic consequences. Mortality and morbidity rates are higher among overweight and obese individuals than lean people. Increased BMI is linked with a greater risk of CHD, hypertension(16), hyperlipidaemia (Appendices, Figure 5), NIDDM and certain cancers. Furthermore, obesity has been recently identified as a major independent risk factor for CHD by the American Heart Association (1997). Modest weight reduction can significantly reduce the risk of these serious health conditions. In addition to the physical consequences on health, obesity creates a massive social burden. Obesity has been described as the "last remaining socially acceptable form of prejudice" (Stunkard & Sobal 1995, p 417). This prejudice does not only exist among the general public but also among the majority of health care professionals. Negative attitudes of health care professionals can seriously impede the treatment of overweight and obese patients (17).

Often over shadowed by the health and social consequences of obesity is the economic cost to society and to the individual. In 1995, for example, in the USA the total economic cost attributable to obesity was estimated at $99 billion (Wolf & Colditz 1998). In several developed countries obesity has been estimated to account for 2-7% of the total health care costs (WHO TRS 894)(18). In addition to the direct costs of obesity are costs in terms of the individuals (including ill health and reduced quality of life (intangible costs)) and society in terms of loss of productivity due to sick-leave and premature pensions (indirect costs). Prevention is clearly more cost effective than treatment, both in terms of economic and personal costs. Health care providers and policy makers need to appreciate the importance of obesity and its prevention, and develop effective policies and programmes to prevent obesity (19).

2.5 Study Benefits/Potential Risk

2.5.1.1 Benefits of Study

The rapidly increasing incidence of obesity in the world makes it imperative to clearly understand the efficacy and mechanisms of action of potential preventive agents derived from the diet. This study carries the mission to determine the clinical efficacy of Tea flower (*Camellia sinensis*) extract from Buds on obesity control.

2.5.1.2 Potential Risk

It has not been reported of the toxicity of Tea flower (*Camellia sinensis*) extract from Buds.
However, various studies were done to examine the toxicity and Potential Risk of *Camellia sinensis* and chakasaponins, and are listed as following:

Experiment	**Duration**	**Route of Delivery**	**Participants**	**Dosage**	**Results**
Safety test in Rats	91 days	Oral	42 male and female rats each	1%(w/w): 0.45~1.21 g/kg/day 1.75%(w/w): 0.84~2.15 g/kg/day 2.5%(w/w):1.11~3.11 g/kg/day	No adverse effect reported.

Ability of Small Nuclear induction test in Male Rats	3 days, 24hrs dosage administration interval	Oral and abdominal injection	6 male ICR - SPF rats	500 g/kg/day 1000 g/kg/day 2000 g/kg/day	No adverse effect reported. None of the results shows the ability of small nuclear induction of the sample.
Oral test in Human	30 days	Oral (6 pills/day)	40 women	100 mg/day 300 mg/day 1000 mg/day	No Adverse effect reported.

In this study, all subjects will be completely monitored for safety parameters and health condition. All AEs will be reported immediately to the Chung Shan Medical University Hospital Institutional Review Board (IRB). Please refer to 7.1 Safety parameters for parameters that will be monitored.

2.6 GCP Compliance

This study will be conducted in compliance with the protocol approved by the Institutional Review Board (IRB) , and according to Good Clinical Practice standards. Significant deviation from the protocol will be reported to the IRB for approval.; Except where it may be necessary to eliminate an immediate hazard to a research subject. In such case, the deviation will be reported to the IRB as soon as possible, Minor deviations will be noted in the study record.

2.7 Reference Literature

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3 Study Objective

The extract from the flower buds of *Camellia sinensis* cultivated in Fujian Province showed inhibitory effects on body weight gain and the weight of visceral fats in high-fat diet-fed mice and/or Tsumura Suzuki Obese Diabetic (TSOD) mice. A suppressive effect of the extract on food intake was suggested to contribute to the anti-obesity effect. The study is designed to investigate the clinical anti-obesity effects of the extract from the flower buds of *C. sinensis*, in combination with EGCG and Nutriose.

4 Study Design

4.1 Primary Objective

In this study we are investigating the anti-obesity effects of the extract from the flower buds of *C. sinensis*. The testing sample is orally administrated to participants with simple obesity syndrome, the efficacy parameters of body weight and body fat percentage and other parameters, as well as adverse effects to the health individual is screened during the trial, significant difference is shown and no adverse effect reported.

4.2 Study Design

Double blind, randomised, placebo controlled trail. Participants are randomized into treatment group and placebo group; prior to intervention and allocation after assess eligibility. Treatment group will be administrated 300mg/day and follow the dosage and regimen prescribed in the study. Investigational product is administrated for 12 weeks. Factors include age; sex, diet, and exercise behaviour are considered with group size greater than 50 participants in each group.

•Number of subjects will be monitored at both stages: 1. Enrolment. 2. End of the trial.

•Outcome measures recorded at baseline, month 1, 2, & 3.

•Exercise level will be assessed using Personal Wellness Profile (PWP question questionnaire), by self-reporting as mild, moderate, or vigorous;

•Aerobic capacity of participants will be assessed using submaximal bike test, with VO2 max measured using Astrand and Rythming method at pre and post stage of study.

Please refer to Appendices II for the protocol and detailed instruction for submaximal bike test.

•Dietary adherence will be measured as following:

•3 day dietary record at baseline, month 1, 2, 3. Macronutrient intake measured including: kcal, protein, carbohydrate, total fat, saturated fat, cholesterol, fiber. Mean self-reported dietary adherence will be assessed by a 0-10 scale.

•Subjects are requested to maintain their regular dietary habits as well as exercise habits, to reduce bias factors.

•Assessment of subject diet & exercise habits at monthly visit using a scale of 0-10. Any changes in diet, medication, hospitalization, exercise or adverse affects are to be reported during the visit.

•Supplement adherence will be measured. This can be discussed at monthly visit and bottles will be collected and returned, unconsumed remaining pills counted at lab visits.

Medical Laboratory Tests will be conducted and listed as following:

- Total cholesterol, HDL(High density lipoprotein), LDL(Low density lipoprotein), TG(Triglyceride), Total/HDL ratio, LDL/HDL ratio.
- Insulin
- ALT(GPT)
- AST(GOT
- GGT
- Leptin*
- Ghrelin*
- Secretin - CCK*(Secretin-cholecystokinin test)
- Adiponectin*
- Inflammatory markers (i.e., CRP, TNF-α, IL-6, IL-1)*
- Cortisol
- Urine analysis
- Total protein
- Nitrogen*
- Creatinine

SCHEDULE OF EVENTS

Evaluation/ Procedure	*Registration*	*Baseline*	*Week 2*	*Week 4*	*Week 6*	*Week 8 or*	*Week 10*	*Week 12 or early termination*	*Follow Up Visit*(To be discussed with PI)*
Informed Consent	*X*								
Medical History & Safety Screening	*X*								
Assess Eligibility	*X*	*X*							
Vital Signs/ Height and Weight*		*X*	*X*	*X*	*X*	*X*	*X*	*X*	
*Medical Laboratory Testes(Blood)**		*X*		*X*		*X*		*X*	
X-Rays, EKG, Abdominal Ultrasound.		*X*						*X*	
Study Evaluations/ Assessments		*X*		*X*		*X*		*X*	
Dispense Study Agent		*X*	*X*	*X*	*X*	*X*	*X*		
Collect & Review Study Agent and Record			*X*	*X*	*X*	*X*	*X*		
Adverse Events			*X*	*X*	*X*	*X*	*X*	*X*	*X*
*Telephone Contact**			*X*		*X*		*X*		*X*

Procedure for SCHEDULE OF EVENTS

Informed consents:
Enrolment stage of the trial, where participants will be gathered and informed for all relevant information for the trial, after all information has been explained and all questions from participants are answered; consent will be obtained from the participants to join the trail.

Medical History and Screening:
Participants are proceeding to medical history and screening, the eligibility will be assessed by Medical Doctors and is done in the clinic, including assessments of medical history, pregnant condition, and appropriate birth control, if necessary.

Assess eligibility
The assessment of eligibility will be assessed by Medical Doctors and including assessments of medical history, pregnant condition, and appropriate birth control if necessary, as well as safety measures.

***Vital Signs, Body weight/Height**

1. Body temperature
   Checking body temperature is to solicit any signs of systemic infection or inflammation in the presence of a fever (temp > 38.5°C or sustained temp > 38°C), or elevated significantly above the individual's normal temperature. Other causes of elevated temperature include hyperthermia. Temperature depression (hypothermia) also needs to be evaluated. Defined as below 36.5°C. Temperature is commonly considered to be a vital sign most notably in a hospital setting.

2. Pulse rate (or heart rate)

   Normal pulse rates at rest, in beats per minute (BPM) (27).

newborn (0-3 months old)	infants (3 — 6 months)	infants (6 — 12 months)	children (1 — 10 years)	children over 10 years & adults, including seniors	well-trained adult athletes
100-150	90–120	80-120	70–130	60–100	40–60

The pulse rate can be used to check overall heart health and fitness level. Generally lower is better, but bradycardias can be dangerous. Symptoms of a dangerously slow heartbeat include weakness, loss of energy and fainting. (28.)

Abnormal pulse rate will be judged by medical doctor in correspond with EKG.

3. Blood pressure

   The blood pressure will be monitored; if a threshold measurement of Blood pressure greater $\geq$ 140 mm Hg for systolic or $\geq$ 90 mm Hg diastolic during seated are observed during the trial, the participant will be excluded from the trail and arrangement for adequate medical attention will be made.

4. Respiratory rate

   Average respiratory rate reported in a healthy adult at rest is usually given as 12-18 breaths per minute (V_f)(30, 31).

***Medical Laboratory Testes (Blood)**

- Total cholesterol, HDL (High density lipoprotein), LDL (Low density lipoprotein), TG (Triglyceride), Total/HDL ratio, LDL/HDL ratio.
- Insulin
- ALT
- AST
- GGT
- Leptin*
- Ghrelin*
- Secretin - CCK*(Secretin-cholecystokinin test)
- Adiponectin*
- Inflammatory markers (i.e., CRP, TNF-α, IL-6, IL-1)*
- Cortisol
- Urine analysis

- Total protein
- Nitrogen*
- Creatinine

•Please note that medical laboratory tests marked with Asterisk are not included in the Taiwan National Health Insurance and Clinical regulatory, additional medical laboratory kits needs to be purchased in order to conduct the tests.

***Study Evaluations/ Assessments**

Major Parameters including:

- waist circumference
- Hipline
- BMI
- Standard weight
- Overweight degree.
- Subjects will be assessed using Personal Wellness Profile (PWP question), questionnaire of Exercise level by self-reporting as mild, moderate, or vigorous;
- 3 day dietary record, Macronutrient intake measured including: kcal, protein, carbohydrate, total fat, saturated fat, cholesterol, fibre. Mean self-reported dietary adherence will be assessed by a 0-10 scale.
- Assessment of subject diet & exercise habits using a scale of 0-10. Any changes in diet, medication, hospitalization, exercise or adverse affects are to be reported during the visit.

***Review Agent Diary/Record**

The agent diary/record will be handed out at the previous visit.

Follow-up period*

Participants will be followed for 2 weeks.

Telephone Contact*

- Supplement adherence will be measured.
- Dietary adherence will be measured as following:
- 3 day dietary record at baseline, month 1, 2, 3. Macronutrient intake measured including: kcal, protein, carbohydrate, total fat, saturated fat, cholesterol, fiber. Mean self-reported dietary adherence will be assessed by a 0-10 scale.
- Dietary adherence will be measured.

4.3 Randomization

The trail is designed to be double-blinded, randomized control trial.

A researcher from Chung Shan Medical University Hospital will perform the randomization procedures by allocating participants to groups according to a computer generated random list.

4.4 Treatment and Dosage

Subjects from treatment group received 300mg per day for at 12 weeks duration.
Dosage: 300mg/capsule, QD, AC.(quaque die, ante cibum). 30 mins before meal.
Subjects form placebo group are administrated with the same dosage of placebo.

4.5 Study Duration

The Study Duration will be a minimum of 12 weeks. Two month period is required for IRB submission and revision procedure including enrolment.

4.6 Discontinuation criteria

Subjects are asked to report immediately if any of these following effects occur: Nausea, diarrhea, upset stomach, headache, and dizziness.
If any of these effects persist the subjects will be asked to stop regimen immediately: Fast/irregular heartbeat, mental/mood changes (e.g., nervousness, confusion), trouble sleeping, restlessness, shakiness (tremor), seizures.
A very serious allergic reaction to the research agent is rare. However, subjects are instructed to report and seek immediate medical attention where appropriate medical counselling will be organized if any of the following symptoms is observed of a serious allergic reaction: rash, itching/swelling (especially of the face/tongue/throat), severe dizziness, trouble breathing.

4.7 Accountability procedures of the investigational products

Supplement adherence will be measured. This can be discussed at monthly visit and bottles will be collected and returned at baseline, week 2, 4, 6, 8, 10 and 12.
Unconsumed remaining pills counted at lab visits.

4.8 Data Collection

Participant data will be collected using protocol-specific case report forms (CRF) developed from the standard set of [Chung Shan Medical University Hospital IRB's CRF Templates]. The approved CRFs will be used in the study application. Site staff will enter data into the CRF. Amended CRFs will be submitted to the IRB for review and approval.

5 Participant's enrolments and exclusion.

5.1 Inclusion criteria

Participants with the simple obesity syndrome, Subjects Age 18-65, male and female Overweight or obese with recognized standard of - BMI =30 or total fat percentage >25 % for males and >30% for females.

5.2 Exclusion criteria

•Patients with liver, heart, renal disease and mental illness will be exclude from the trial.
•Has intake any substance that is related to the investigational products, and is likely to influence the judgment of study results.
•Lost to follow-up, non-compliance, concomitant medication.
• Presence of cardiovascular disease, cancer, diabetes mellitus, inflammatory bowel disease, or any other chronic health condition identified from the findings of the interview.
•Blood pressure greater $\geq$ 140 mm Hg systolic or $\geq$ 90 mm Hg diastolic during seated, resting measurement on two consecutive occasions during visit 1.
•Fasting plasma triglycerides > 200 mg/dl.
•Use of lipid lowering medications (includes TCM and dietary supplements)
•Use of blood pressure lowering medications (includes TCM and dietary supplements)
•Pregnant or lactating women, or women of child-bearing potential unwilling to use a medically approved form o birth control.
•History or current abuse of drug f
•s or alcohol, or intake > 2 alcoholic beverages per day.
•Participation in another clinical trial within 30 days of enrollment into the study.

5.3 Clinical End Point

Participants may go 'off-study' for the following reasons: the protocol intervention and any protocol-required follow-up period is completed, adverse event/serious adverse event, lost to follow-up, non-compliance, concomitant medication, medical contraindication, withdraw consent, death.

6 Efficacy assessments

6.1 Efficacy parameters

Major Parameters including:

- Body weight
- Height
- waist circumference
- Hipline
- BMI
- Standard weight
- Overweight degree.

Body weight measurement:
Single calibrated scale (i.e., Detecto) is used, with light clothes and no shoes. BMI is calculated.

Body fat percentage measurement:
Body composition using BIA measurement.
Subcutaneous fat level is measured using B-mode ultrasound or clippers at 4 measurement points:

- The midpoint of lower, outer side of right deltoid.
- Right Scapular
- Navel, 3 centimetre to the right.
- Right anterior, superior iliac spine

Waist circumference measurement:
2 readings at the umbilicus with a spring calibrated tape measure

- Blood pressure measured using an automated instrument, measuring condition fixed: subjects seated, mean of 1 measurement reading in each arm.

BMI Calculations:
Metric Units: BMI = Weight (kg) / (Height (m) x Height (m))
English Units: BMI = Weight (lb) / (Height (in) x Height (in)) x 703

$$\text{Overweight degree}(\%) = \frac{\text{Actual Weight} - \text{Standard weight}}{\text{Standard weight}} \times 100\%$$

Standard Weight

6.0 Assessment of efficacy parameters

The efficacy will be assessed under the following parameters and condition:

- For comparison of treatment group with control group, body fat percentage reduction report is significant
- At least two out of four subcutaneous fat level checkpoints decrease.
- The waistline or hip circumference of the subjects in the test groups is reduced significantly ($P < 0.05$).
- Exercise tolerance is not reduced;
- No adverse effects reported on the subject's physical health, weight-control effects caused by diets and exercise have been eliminated.

7 Safety assessments

7.1 Safety parameters

- Lipid
- Panel
- Insulin
- Leptin
- Ghrelin
- CCK
- Adiponectin
- inflammatory markers
- Cortisol

•Life condition including mental, sleep, diet, Excretion, blood pressure screening.
•Laboratory medical test including urine, faeces examination.
•Liver, renal function, abdominal ultrasound prior to the study commence, children does not need such assessments.
•Blood uric acid, ketone body.
•Subjects will be assessed using PWP questionnaire of Exercise level by self-reporting as mild, moderate, or vigorous; aerobic capacity is assessed use Submax test -bike or treadmill, VO2Max(L/mins) is measured using Astrand and Ryhming method.

Medical Laboratory Tests will be conducted and listed as following:

- Total cholesterol, HDL(High density lipoprotein), LDL(Low density lipoprotein), TG(Triglyceride), Total/HDL ratio, LDL/HDL ratio.
- Insulin
- ALT
- AST
- GGT
- Leptin*
- Ghrelin*
- Secretin - CCK*(Secretin-cholecystokinin test)
- Adiponectin*
- Inflammatory markers (i.e., CRP, TNF-α, IL-6, IL-1)*
- Cortisol
- Urine analysis
- Total protein
- Nitrogen*
- Creatinine

7.0 SAE Report

The TCI Co., Ltd and Chung Shan Medical University Hospital will report SAEs to the IRB of Chung Shan Medical University Hospital according to the Serious Adverse Event Reporting Procedures and Guidelines as posted in the Clinical Trials Resource on the website of Chung Shan Medical University Hospital IRB. SAE reports to the IRB within 48 hours of learning of the event using the paper SAE form.

7.1 Definition of SAE

All adverse events that occur after the informed consent is signed (including run-in) must be recorded on the adverse event CRF (paper and/or electronic) whether or not related to study agent. AE Data Elements including:

- AE reported date
- AE Verbatim Term
- CTCAE Term (v 3.0)
- Event onset date and event ended date
- Severity grade
- Attribution to study agent (relatedness)
- Whether or not the event was reported as a Serious Adverse Event (SAE)
    - Action taken with the study agent
    - Outcome of the event

- Comments

The adverse event is identified using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. The CTCAE provides descriptive terminology and a grading scale for each adverse event listed.

AEs will be assessed according to the CTCAE grade associated with the AE term. AEs that do not have a corresponding CTCAE term will be assessed according to their impact on the participant's ability to perform daily activities as follows:

Grade	*Severity*	*Description*
1	***Mild***	• ***Barely noticeable, does not influence functioning*** • ***Causing no limitations of usual activities***
2	***Moderate***	• ***Makes participant uncomfortable, influences functioning*** • ***Causing some limitations of usual activities***
3	***Severe***	• ***Severe discomfort, treatment needed*** • ***Severe and undesirable, causing inability to carry out usual activities***
4	***Life threatening***	• ***Immediate risk of death*** • ***Life threatening or disabling***
5	***Fatal***	• ***Causes death of the participant***

The possibility that the adverse event is related to study agent will be classified as one of the following: not related, unlikely, possible, probable, definite.

DEFINITION of Serious Adverse Events: ICH Guideline E2A and GCP of Taiwan define serious adverse events as those events, occurring at any dose, which meet any of the following criteria:

- Results in death
- Is life threatening (Note: the term life-threatening refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe).

- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital abnormality/birth defect
- Events that may not meet these criteria, but which the investigator finds very unusual and/or potentially serious, will also be reported in the same manner.

7.2 SAE Follow-up

All AEs, including lab abnormalities that in the opinion of the investigator are clinically significant, will be followed according to good medical practices and documented as such. Site staff should send follow-up reports as requested when additional information is available. Additional information should be entered on the IRB of Chung Shan Medical University Hospital of SAE form in the appropriate format. Follow-up information should be sent to Chung Shan Medical University Hospital IRB as soon as possible according to the Chung Shan Medical University Hospital IRB's Serious Adverse Event Reporting Procedures and Guidelines.

8 Statistical Analysis

8.1 Statistical Methods

T – Test is performed and 2-sided type I error of 5% to detect 2% weight change. T – pair test and F – Test is performed for group data. Variance needs to be converted for non-positive skewed and Heterogeneity of variance observed, when positive skewed is satisfied, t-test is performed on converted data, if the converted data is yet unable to satisfy positive skewed, rank sum test can be performed, especially for data with CV>50%.

8.0 Level of Significance)

Level of significance is defined as $P<0.05$.

9 Ethics/Protection of Human Subjects

This study will be conducted according to Taiwan and international standards of Good Clinical Practice for all studies. Applicable government regulations and Chung Shan Medical University Hospital Institutional Review Board (IRB) Hospital research policies and procedures will also be followed.

This protocol and any amendments will be submitted to the Chung Shan Medical University Hospital Institutional Review Board (IRB) for formal approval to conduct the study. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB. The formal consent of a subject, using the IRB-approved consent form, will be obtained before that subject is submitted to any study procedure. This consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

10 Data collection and maintenance

Clinical records for all participants, including CRFs, all source documentation (containing evidence to study eligibility, history and physical findings, laboratory data, results of consultations, etc.), as well as IRB records and other regulatory documentation will be retained by the Protocol Lead Investigator in a secure storage facility in compliance with Taiwan regulations and guidance.

11 Confidentiality

TCI CO, Ltd. And Amyway China Company Reserves copyrights of the data. Any distribution, publication, citation or copying of the document requires official authorization from TCI CO, Ltd.and the Amway China company.

Declaration of Helsinki

11.1.1 WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53th WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added)
55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added)
59th WMA General Assembly, Seoul, October 2008

A. INTRODUCTION

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

   The Declaration is intended to be read as a whole and each of its constituent paragraphs should not be applied without consideration of all other relevant paragraphs.

2. Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human subjects to adopt these principles.

3. It is the duty of the physician to promote and safeguard the health of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

4. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."

5. Medical progress is based on research that ultimately must include studies involving human subjects. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.

6. In medical research involving human subjects, the well-being of the individual research subject must take precedence over all other interests.

7. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best current

interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

8. In medical practice and in medical research, most interventions involve risks and burdens.

9. Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and rights. Some research populations are particularly vulnerable and need special protection. These include those who cannot give or refuse consent for themselves and those who may be vulnerable to coercion or undue influence.

10. Physicians should consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

11. It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.

12. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

13. Appropriate caution must be exercised in the conduct of medical research that may harm the environment.

14. The design and performance of each research study involving human subjects must be clearly described in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. The protocol should describe arrangements for post-study access by study subjects to interventions identified as beneficial in the study or access to other appropriate care or benefits.

15. The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration. The committee must have the right to monitor ongoing studies. The

researcher must provide monitoring information to the committee, especially information about any serious adverse events. No change to the protocol may be made without consideration and approval by the committee.

16. Medical research involving human subjects must be conducted only by individuals with the appropriate scientific training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional. The responsibility for the protection of research subjects must always rest with the physician or other health care professional and never the research subjects, even though they have given consent.

17. Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.

18. Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.

19. Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.

20. Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.

21. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research subjects.

22. Participation by competent individuals as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.

23. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity.

24. In medical research involving competent human subjects, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as

to the methods used to deliver the information. After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

25. For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee.

26. When seeking informed consent for participation in a research study the physician should be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent should be sought by an appropriately qualified individual who is completely independent of this relationship.

27. For a potential research subject who is incompetent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the population represented by the potential subject, the research cannot instead be performed with competent persons, and the research entails only minimal risk and minimal burden.

28. When a potential research subject who is deemed incompetent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.

29. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research population. In such circumstances the physician should seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research should be obtained as soon as possible from the subject or a legally authorized representative.

30. Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should adhere to accepted guidelines for ethical reporting. Negative and

inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

31. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

32. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:
    - The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or
    - Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the patients who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.

33. At the conclusion of the study, patients entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.

34. The physician must fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never interfere with the patient-physician relationship.

35. In the treatment of a patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.

Appendices.

Appendices I

11.2 Tables

11.2.1 Table 1: WHO standard classification of obesity

	BMI	Risk of co-morbidities
Normal BMI	18.5-25.0	average
Overweight:		
Pre-obese	25.0-29.9	increased
Obesity class I	30.0-34.9	moderate
Obesity class II	35.0-39.9	severe
Obesity class III	□ 40	very severe

(WHO 1997)

11.2.2 Table 2: Sex-specific waist circumferences for 'increased risk' and 'substantially increased risk' of metabolic complications associated with obesity in Caucasians

	Risk of obesity-associated metabolic complications	
	Increased	Substantially increased
Men	□ 94 cm	□ 102 cm
Women	□ 80 cm	□ 88 cm

Note: The figure are population-specific and the relative risk also depends on levels of obesity (BMI) and other risk factors for CVD and NIDDM) (WHO report 1997)

11.2.3 Table 3: Prevalence of overweight* children aged 6 to 8 year old

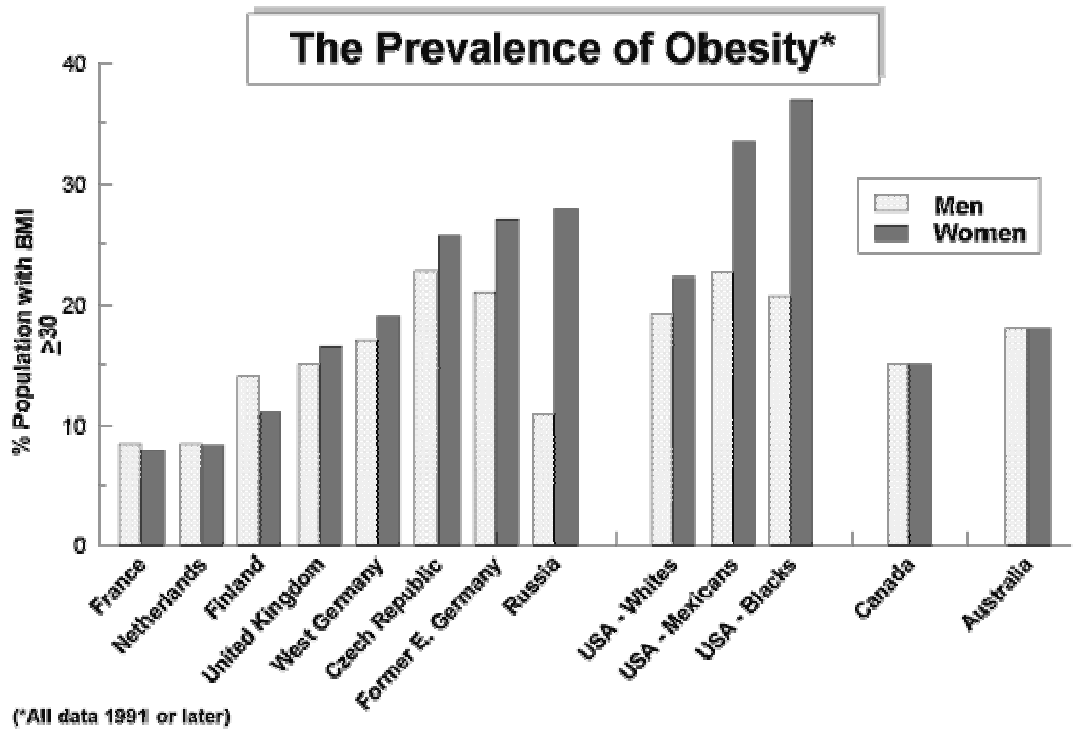
	USA 1988-91	China 1993	Russia 1994-5	South Africa 1994	Brazil 1989
Girls	24.2	12.2	17.8	20.3	10.5
Boys	21.3	14.1	25.6	25.0	12.8

Popkin et al 1996

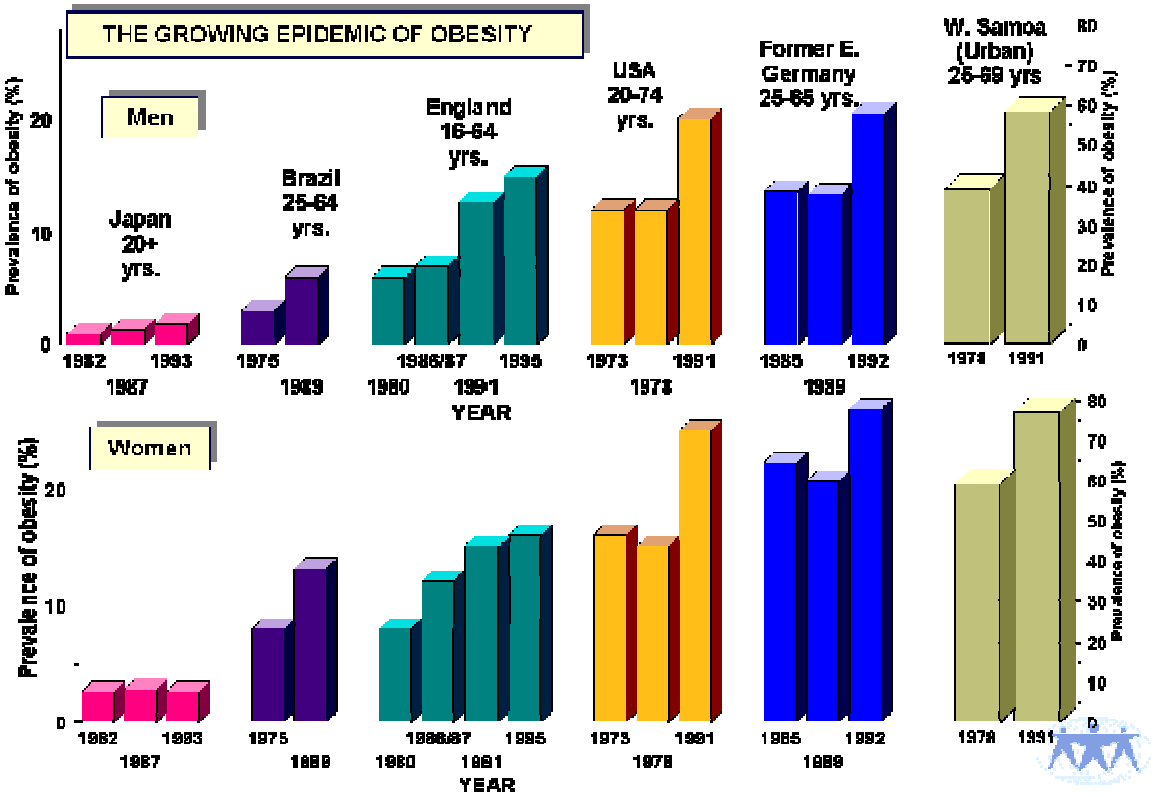
(*defined as BMI higher than the US reference NHES 85th percentile)

11.3 Figures

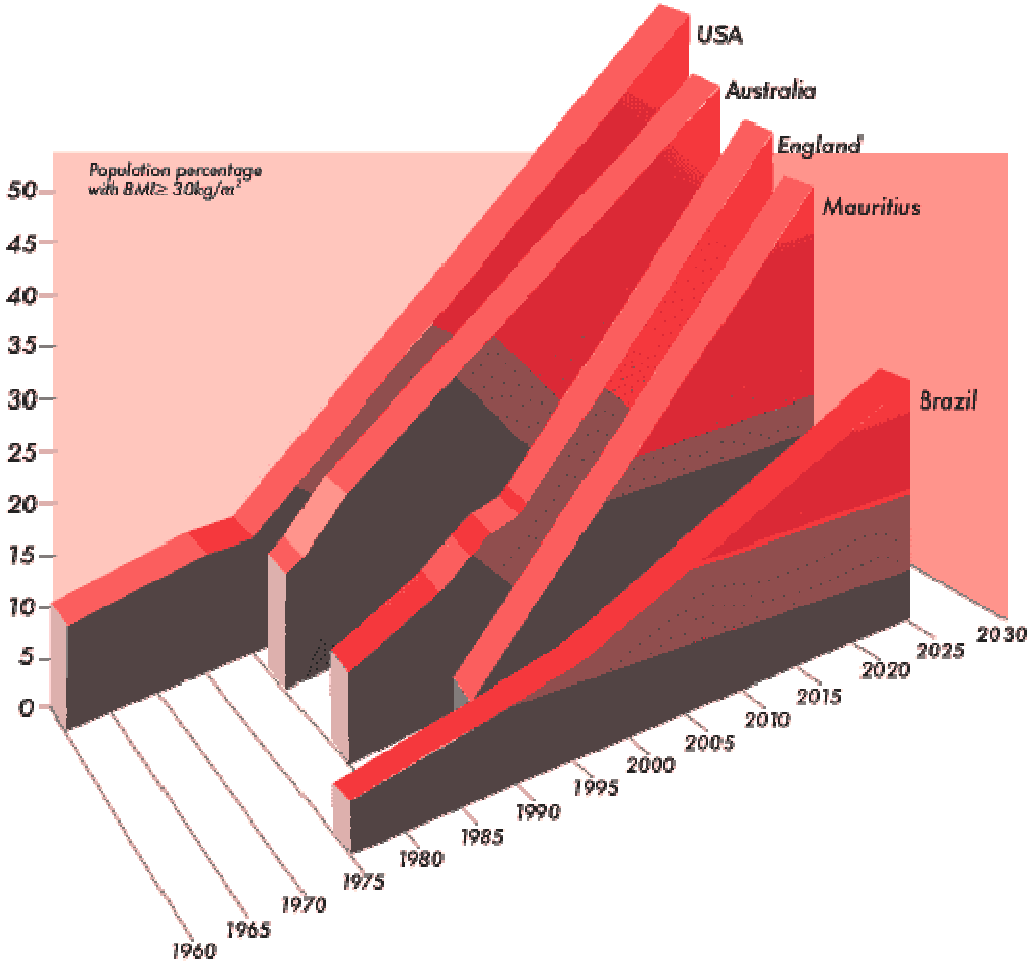
11.3.1 Figure 1: Examples of the prevalence of obesity in adults throughout the world



11.3.2 Figure 2: The increasing prevalence of obesity in adults world-wide.

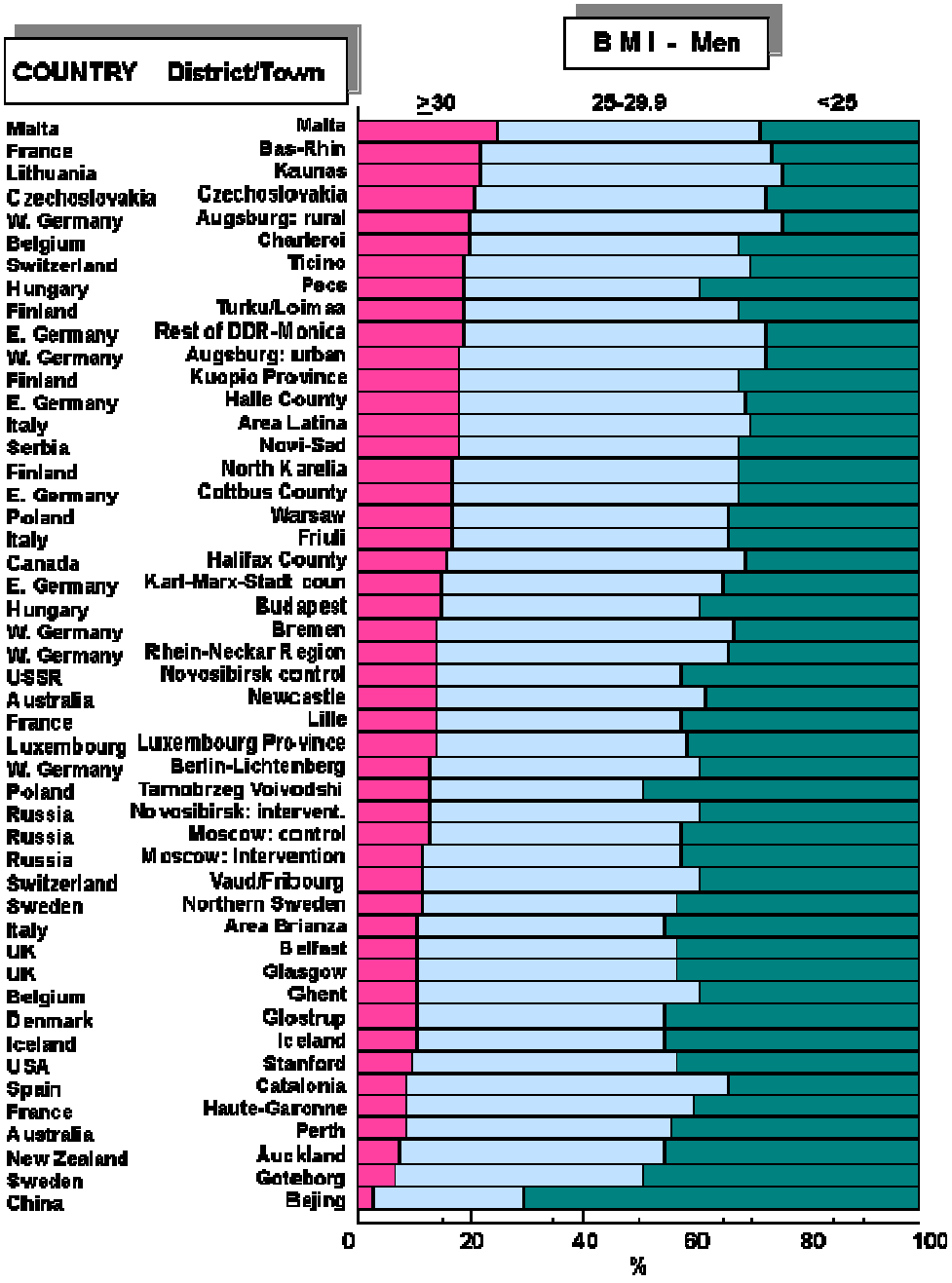


11.3.3 Figure 3: Projected prevalence of obesity in adults by 2025

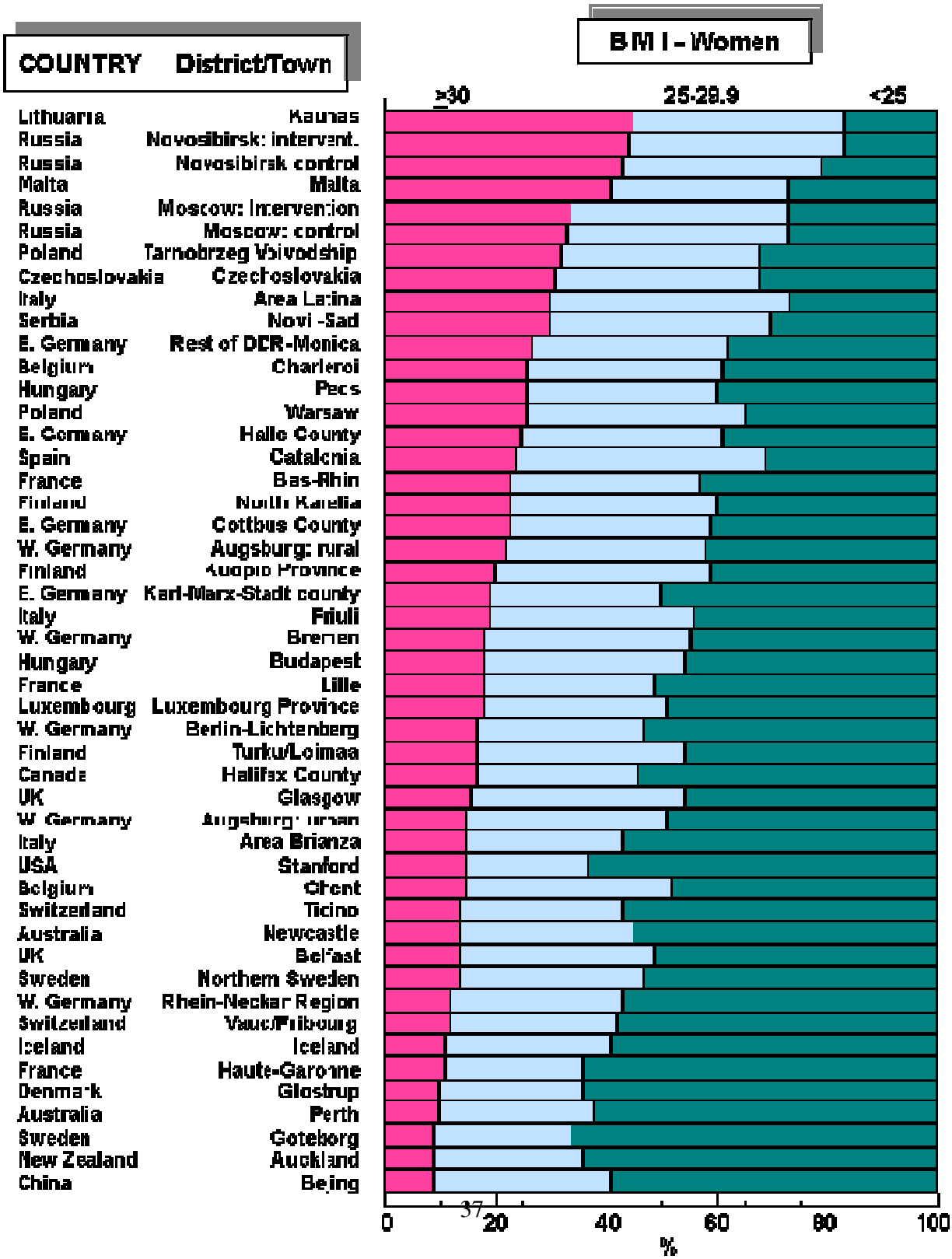


11.3.4 Figure 4: BMI distributions: proportion of men and women classified as obese, overweight and normal weight.

Body Mass Index distribution: age-standardized proportions of selected categories in MONICA populations, age group 35-64 years.

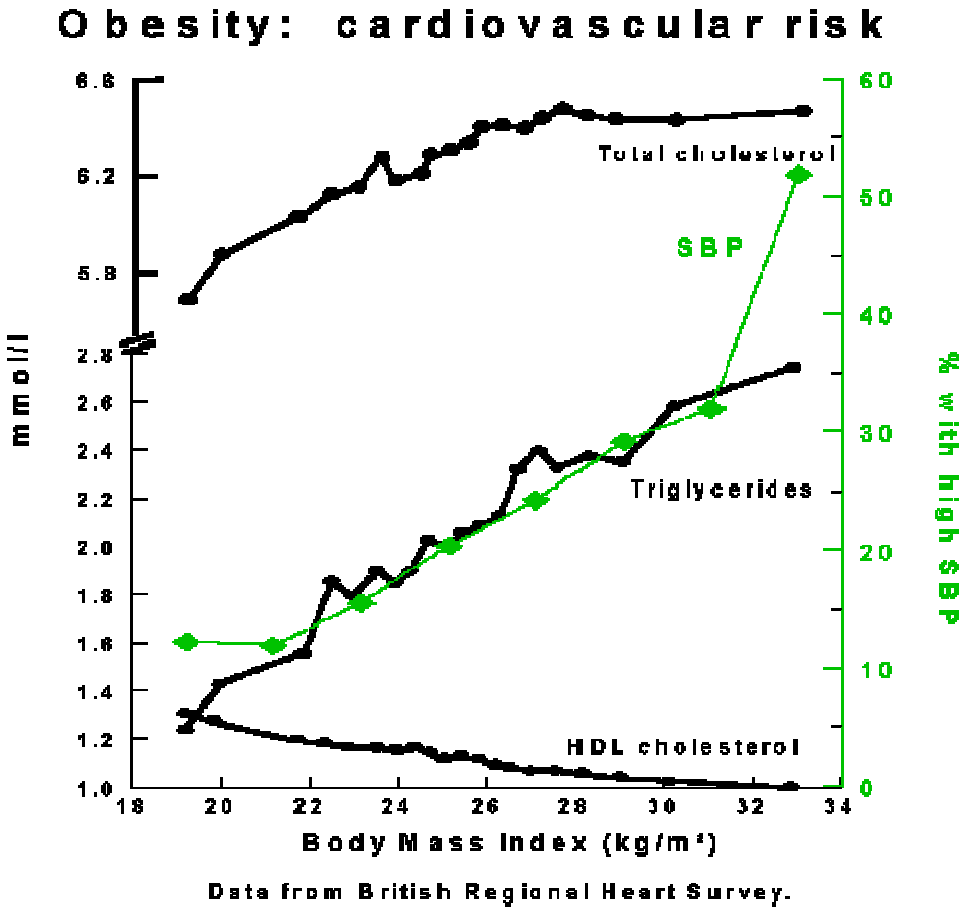


Body Mass Index distribution: age-standardized proportions of selected categories in MONICA populations age group 35-64 years.

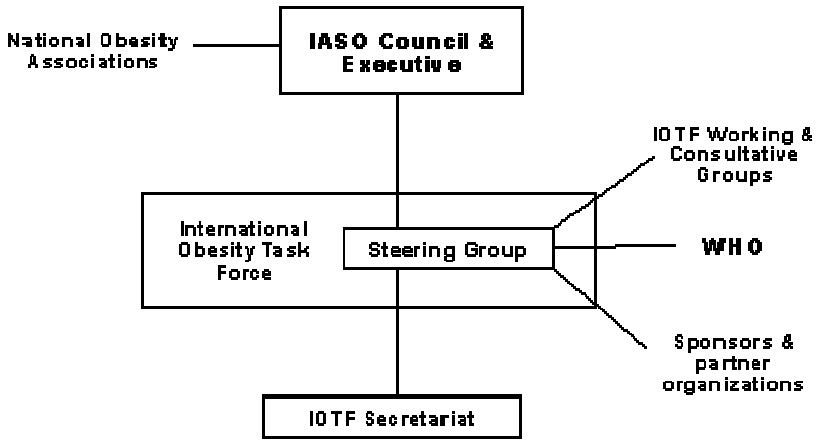


Note: Age standardised proportions from MONICA populations (age 35-64 years), data collected 1983-1986.

11.3.5 Figure 5: Relationship between BMI and cardiovascular risk factors



11.3.6 Figure 6: The organisational structure of the IOTF



12 Appendices II

Sub maximal Bike Test:
The Astrand 6 minute protocol and instruction:

Testing and measurement are the means of collecting information upon which subsequent performance evaluations and decisions are made but in the analysis we need to bear in mind the factors that may influence the results.

The Astrand 6 minute Cycle Test was devised by Astrand P.O. in 1956.

12.1.1 Objective

The objective of this test is to monitor the athlete's VO2max.

12.1.2 Materials

To undertake this test these materials are required:

- Cycle ergometer
- Heart rate monitor
- Weighing Scales
- Stopwatch
- Assistant

12.1.3 How to conduct the test

- The assistant weighs and records the athlete's weight
- The athlete warms up for 10 minutes
- The assistant sets the initial cycle work rate kg-m/min (kilogram-force meter/minute)as follows:
    - Male under 35 – 125
    - Male 35-55 – 115
    - Male over 55 – 85
    - Female under 35 – 115
    - Female 35-55 – 85
    - Female over 55 – 60

  1 watt is equal to 3600 J/hr, or 6.11829727787 kg-m/min

- This setting should raise the athlete's heart rate to 130-160bpm after 2 minutes cycling at 60rpm
- The assistant gives the command "GO" and starts the stopwatch
- The athlete pedals at 60rpm for 6 minutes whilst maintaining their heart rate between 130-160bpm
- The assistant records the athlete's heart rate each minute

- After 2 minutes - if the athlete's heart rate is not in the target range of 130-160bpm then the assistant adjusts the work rate wattage accordingly
- The assistant stops the test after 6 minutes and records the final work rate wattage

12.1.3.1 Convert rpm to km/hr

The unit is converted using formulation tools provided by the website.**Assessment**

The following calculator utilises a series of polynomial equations based on the Astrand-Ryhming Nomogram (Astrand 1954) **(28.)**

To obtain an estimate of your Vo2 (L) and VO2max (ml/kg/min) please enter your, gender, weight, pulse rate and work rate (kg-m/min) and then select the 'Calculate' button.

Gender Male

Weight Pounds

Pulse Rate bpm (Range: 130-160)

Work Rate kg-m/min (Range: Male 450-900 & Female 300-750)

Estimated VO2 - L

Estimated VO2max - ml/kg/min

If the estimated values are 0.00 then the values you entered are outside the specified ranges and you will need to use the Astrand-Ryhming Nomogram to obtain your estimates.

For an analysis of your VO2max score see the VO2max page.

12.1.5 Analysis

Analysis of the test result is by comparing it with the athlete's previous results for this test. It is expected that, with appropriate training between each test, the analysis would indicate an improvement in the athlete's VO2max.

12.1.5.1 Target Group

This test is suitable for endurance athletes and players of endurance sports (e.g. football, rugby) but not for individuals where the test would be contraindicated.

12.1.5.2 Reliability

Test reliability refers to the degree to which a test is consistent and stable in measuring what it is intended to measure. Reliability will depend upon how strict the test is conducted and the individual's level of motivation to perform the test. The following link provides a variety of factors that may influence the results and therefore the test reliability.

12.1.5.3 Validity

Test validity refers to the degree to which the test actually measures what it claims to measure and the extent to which inferences, conclusions, and decisions made on the basis of test scores are appropriate and meaningful. This test provides a means to monitor the effect of training on the athlete's physical development. There are published VO2max tables and the correlation to actual VO2max is high. For an assessment of your VO2max see the VO2max normative data tables.

12.1.6 Advantages

- Minimal equipment required
- Simple to set up and conduct

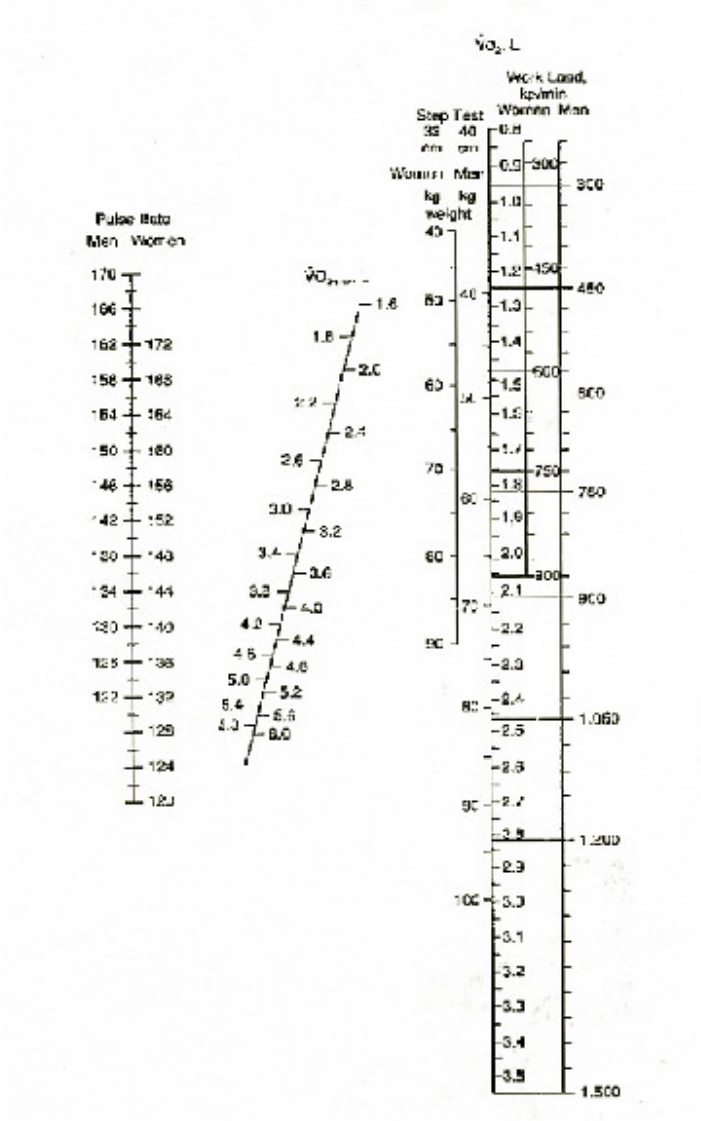
12.1.7 Disadvantages

- Specialist equipment required
- Assistant required to administer the test

12.1.8 Referenced Material

ASTRAND, P.O. & RYHMING, I. (1954) A nomogram for calculation of aerobic capacity (physical fitness) from pulse rate during submaximal work. *J Appl Physiol*, 7, p. 218-221.

Modified Astrand-Ryhming Nomogram



Reference: ACSM Guidelines for Exercise Testing and Prescription, Edition 5

Figure.. The Astrand-Ryhming Nomogram.

13 Appendices III

Protocols for Medical Laboratory Tests.

Please refer to the following page, double click on the page to open.

Uscn Life Science Inc.
www.uscnk.us; www.uscnk.cn; www.uscnk.com

E90802Hu 96 Tests
Enzyme-linked Immunosorbent Assay Kit
For Cholecystokinin (CCK)
Organism: Homo sapiens (Human)
Instruction manual

FOR IN VITRO USE AND RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES

7th Edition (Revised in November, 2011)

[INTENDED USE]

The kit is a competitive inhibition enzyme immunoassay technique for the in vitro quantitative measurement of CCK in human serum, plasma, tissue homogenates, cell culture supernates and other biological fluids.

[REAGENTS AND MATERIALS PROVIDED]

Reagents	Quantity	Reagents	Quantity
Pre-coated, ready to use 96-well strip plate	1	Plate sealer for 96 wells	4
Standard (lyophilized)	2	Standard Diluent	1×20mL
Detection Reagent A (green)	1×120μL	Assay Diluent A (2 × concentrate)	1×6mL
Detection Reagent B (red)	1×120μL	Assay Diluent B (2 × concentrate)	1×6mL
TMB Substrate	1×9mL	Stop Solution	1×6mL
Wash Buffer (30 × concentrate)	1×20mL	Instruction manual	1

[MATERIALS REQUIRED BUT NOT SUPPLIED]

1. Microplate reader with 450 ± 10nm filter.
2. Precision single or multi-channel pipettes and disposable tips.
3. Eppendorf Tubes for diluting samples.
4. Deionized or distilled water.
5. Absorbent paper for blotting the microtiter plate.
6. Container for Wash Solution

[STORAGE OF THE KITS]

1. For unopened kit: All the reagents should be kept according to the labels on vials. The **Standard**, **Detection Reagent A, Detection Reagent B** and the **96-well strip plate** should be stored at -20°C upon receipt while the others should be at 4°C.

2. For opened kit: When the kit is opened, the remaining reagents still need to be stored according to the above storage condition. Besides, please return the unused wells to the foil pouch containing the desiccant pack, and reseal along entire edge of zip-seal.