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	Reason For Issue	approved by all approvers but not cinverted into effective by its own rather showing approved.		

Clinical Protocol

207192

CONFIDENTIAL

SUMMARY INFORMATION

Title:	Impact of a Nutritional supplement on Bone turnover markers in Indian healthy premenopausal women (25-45 yrs; inclusive) after 6 months of intervention: a randomised double blind controlled trial.
Protocol Number:	**207192**
Sponsor:	GlaxoSmithKline Consumer Healthcare (GSKCH) R&D, Plot No.67, Sector 32, Gurgaon 122001 India
Product Name:	Fortified HFD
Development Phase:	NA

Expert Advice Outside of Normal Working Hours:	**Tel:** PPD

Key Protocol Authors:	
PRIMARY CONTACT	PPD ~~MSc.~~

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PRINCIPAL INVESTIGATOR PROTOCOL AGREEMENT PAGE

I confirm agreement to conduct the study in compliance with the protocol and any amendments and according to the current ICH GCP guidelines.

I acknowledge that I am responsible for overall study conduct. I agree to personally conduct or supervise the described study.

I agree to ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations. Mechanisms are in place to ensure site staff receives all appropriate information throughout the study.

I agree to conduct this study in full conformance with the laws and regulations of the country in which the research is conducted and the Declaration of Helsinki.

Investigator Name:	**DR. PRAMOD B UMARJI**
Investigator Qualifications:	**MBBS, MD**
Investigator Signature:	PPD
Date of Signature/ Agreement:	DD/MMM/YYYY

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PROCESS FOR AMENDING THE PROTOCOL

Protocol modifications to ongoing studies which could potentially adversely affect the safety of subjects or which alter the scope of the investigation, the scientific quality of the study, the experimental design, dosages, duration of therapy, assessment variables, the number of subjects treated, or subject selection criteria are considered major/substantial amendments and must be made only after appropriate consultation between an appropriate representative of GSKCH and the investigator.

Details of amendments to the protocols should be recorded on the following page. Protocol modifications must be prepared by a representative of GSKCH. All changes must be justified in the Reason for Amendment section of the following Protocol Amendment Page. Approval of amendments will be made by the original protocol signatories or their appropriate designees.

All major/substantial protocol modifications must be reviewed and approved by the appropriate IRB/IEC in accordance with local requirements, before the revised edition can be implemented.

All non-substantial/ minor/ administrative amendments should be submitted to the IRB/ IEC as per country specific requirements. In some countries pre-approval of a minor amendment is not required and will just be held on file by the sponsor and investigator.

PROTOCOL AMENDMENT PAGE

Details of all amendments should be recorded in the table below. Affected sections should be listed in the table; the actual amendment/ change should be made in the relevant section of the main protocol.

To highlight the change, the following features will be used:

To **add** text: Use of **CAPITAL LETTERS, BOLD AND UNDERLINE**

To **delete** text: Use of Strikethrough e.g. ~~**strikethrough**~~

Amendment No. & New Protocol Version No.	Type of Amendment	Reason for Amendment	Other Documents Requiring Amendment	Section(s) Amended	PI Amendment Agreement Signature & Date
Amendment No.: 01	Non-Substantial/Minor ☐	Site and PI and laboratory detail was not finalized earlier	Informed Consent ☐ Yes ☒ No	Summary information, PI, Site and lab details added	Signature: PPD

Protocol Version No.: 02	Substantial/ Major ☒		Safety Statement ☐ Yes ☒ No CRF ☐ Yes ☒ No		Date: PPD
Amendment No.: 01	Non-Substantial/Minor ☐	Sample life cycle details were not available earlier, now lab provided this	Informed Consent ☒ Yes ☐ No Safety Statement ☐ Yes ☒ No CRF ☒ Yes ☐ No	STUDY ASSESSMENTS AND PROCEDURES, Lab assessment procedure detail added about collection, storage, transport and analysis of HBS	Signature: PPD
Protocol Version No.: 02	Substantial/ Major ☒				Date: PPD

Amendment No. & New Protocol Version No.	Type of Amendment	Reason for Amendment	Other Documents Requiring Amendment	Section(s) Amended	PI Amendment Agreement Signature & Date
Amendment No.: 01	Non-Substantial/Minor ☐	Software updates not available earlier	Informed Consent ☐ Yes ☒ No Safety Statement ☐ Yes ☒ No CRF ☐ Yes ☐ No	STUDY ASSESSMENTS AND PROCEDURES, Dietary assessment detail regarding software to be used for dietary data analysis added	Signature: PPD
Protocol Version No.: 02	Substantial/ Major ☒				Date: PPD
Amendment No.: 01	Non-Substantial/Minor ☒	New product code provided by NPD	Informed Consent ☐ Yes ☒ No Safety Statement ☐ Yes ☒ No CRF ☐ Yes ☒ No	Product information, MFC, new product added	Signature: PPD
Protocol Version No.: 02	Substantial/ Major ☐				Date: PPD

Amendment No.: 01 **Protocol Version No.:** 02	Non-Substantial/Minor ☐ Substantial/ Major ☒	Updated information from NPD received and suggestion received from reviewers to add some information	Informed Consent ☐ Yes ☒ No Safety Statement ☐ Yes ☒ No CRF ☐ Yes ☒ No	Appendix, Product nutritional profile	Signature: PPD Date: PPD
Amendment No.: 01 **Protocol Version No.:** 02	Non-Substantial/Minor ☐ Substantial/ Major ☒	Due to amendments changed required in schedule	Informed Consent ☐ Yes ☒ No Safety Statement ☐ Yes ☒ No CRF ☒ Yes ☐ No	Study schedule updated as per amendments	Signature: PPD Date: PPD
Amendment No. & New Protocol Version No.	**Type of Amendment**	**Reason for Amendment**	**Other Documents Requiring Amendment**	**Section(s) Amended**	**PI Amendment Agreement** **Signature & Date**

Amendment No.: 02 **Protocol Version No.:** 03	Non-Substantial/Minor ☐ Substantial/ Major ☒	Due to regulatory requirement NFT revised, K (Potassium added to NFT) which was not mentioned.	Informed Consent ☒ Yes ☐ No Safety Statement ☒ Yes ☐ No CRF ☐ Yes ☒ No	Section 12.1 Appendix 1 Product Profile	Signature: PPD Date: PPD
Amendment No.: 02 **Protocol Version No.:** 03 03	Non-Substantial/Minor ☒ Substantial/ Major ☐	Model of Weighing machine is changed by manufacturer, new name is used	Informed Consent ☐ Yes ☒ No Safety Statement ☐ Yes ☒ No CRF ☐ Yes ☒ No	6.7 Anthropometric measurement height and weight	Signature: PPD Date: PPD
Amendment No.: 02 **Protocol**	Non-Substantial/Minor ☐ Substantial/ Major	Lab manual updated with new method which is convenient and accurate, 24 hour urine collection not required, spot test for CTX-1, Calcium and Creatinine	Informed Consent ☒ Yes ☐ No Safety Statement ☐ Yes ☒ No	6.9 Lab assessment	Signature: PPD Date:

Version No.: 03 03	☒	will work.	CRF ☒ Yes ☐ No		PPD
Amendment No.: 02	Non-Substantial/Minor ☐	For dietary assessment Portion size is not accurate measure and subjective, use of weighing scale gives accurate amount consume. Portion size is replaced by measured amount	Informed Consent ☐ Yes ☒ No Safety Statement ☐ Yes ☒ No CRF ☐ Yes ☒ No	6.10 Dietary assessment.	Signature: PPD
Protocol Version No.: 03	Substantial/ Major ☒				Date: PPD
Amendment No.: 02	Non-Substantial/Minor ☐	According to latest lab manual Zn and Vit B6 is measured in plasma not in serum	Informed Consent ☐ Yes ☒ No Safety Statement ☐ Yes ☒ No CRF ☐ Yes ☒ No	Section-2 Objectives and End points	Signature: PPD
Protocol Version No.: 03	Substantial/ Major ☒				Date: PPD
Amendment No.:	Non-Substantial/Minor ☐	According to latest lab manual only test run	Informed Consent ☐ Yes ☒ No	Section-6.9 Lab assessment	Signature: PPD

02 **Protocol Version No.:** 03	Substantial/ Major ☒	biomarker lab will be run batches not all because kit can analyze 36 test in one batch.	Safety Statement ☐ Yes ☒ No CRF ☐ Yes ☒ No		Date: PPD
Amendment No.: 03 **Protocol Version No.:** 04	Non-Substantial/Minor ☒ Substantial/ Major ☐	On few places replacement of serum by plasma for testing Vitamin B6 and Zn was missed in previous amendment due to typo	Informed Consent ☐ Yes ☒ No Safety Statement ☐ Yes ☒ No CRF ☐ Yes ☒ No	1. Introduction, objectives and end points 2. 9.3.3 secondary analysis	Signature: PPD Date: PPD
Amendment No.: 03 **Protocol Version No.:**	Non-Substantial/Minor ☒ Substantial/ Major ☐	Hemoglobin measurement was missed from mentioning in schedule of events on visit 2 while it is in procedure	Informed Consent ☒ Yes ☐ No Safety Statement ☐ Yes ☒ No CRF ☐ Yes ☒ No	Schedule of events	Signature: PPD Date: PPD

04					
Amendment No.: 03	Non-Substantial/Minor ☒	Change of study manager and contact number	Informed Consent ☐ Yes ☒ No Safety Statement ☐ Yes ☒ No CRF ☐ Yes ☒ No	Summary details	Signature: PPD
Protocol Version No.: 4 04	Substantial/ Major ☐				Date: PPD
Amendment No.: 03	Non-Substantial/Minor ☒	Storage temperature for HBS is updated as per revised stability data shared by lab.	Informed Consent ☒ Yes ☐ No Safety Statement ☐ Yes ☒ No CRF ☐ Yes ☒ No	6.9 Lab assessment	Signature: PPD
Protocol Version No.: 04	Substantial/ Major ☐				Date: PPD
Amendment No.: 03	Non-Substantial/Minor ☒	Time duration of SAEs collection was not matching between AEs recording and SAEs recording section	Informed Consent ☐ Yes ☒ No Safety Statement	7.2 Recording of AEs and SAEs	Signature: PPD

Protocol Version No.: 04	Substantial/ Major ☐	which is updated now	☐ Yes ☒ No CRF ☐ Yes ☒ No		Date: PPD
Amendment No.: 03	Non-Substantial/Minor ☒	Time of sample storage changed from 14 days after last sample analysis to 14 days after each sample analysis to align with lab contract	Informed Consent ☐ Yes ☒ No Safety Statement ☐ Yes ☒ No CRF ☐ Yes ☒ No	6.9 Lab assessment	Signature: PPD
Protocol Version No.: 04	Substantial/ Major ☐				Date: PPD
Amendment No.: 03	Non-Substantial/Minor ☒	Clarification regarding product administration upto visit 6 was missed	Informed Consent ☐ Yes ☒ No Safety Statement ☐ Yes ☒ No CRF ☐ Yes ☒ No	Schedule of event Study design (visit 6)	Signature: PPD
Protocol Version No.: 04	Substantial/ Major ☐				Date: PPD
Amendment No.:	Non-Substantial/Minor ☒	Terminology 'Product compliance report' replaced with 'Subject diary card for	Informed Consent ☐ Yes ☒ No	Section 5.5 Product	Signature: PPD

03		dose compliance'	Safety Statement ☐ Yes ☒ No CRF ☐ Yes ☒ No	compliance	
Protocol Version No.: 04	Substantial/ Major ☐				Date: PPD

	Document Name	Protocol CCI 207192		
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SCHEDULE OF EVENTS

Procedure/ Assessment		Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6
		Screening	Baseline Visit (+7- to 10 days post screening)	Follow up visit (45 days +/- 7 days from visit2)	Midline Visit (90 days from baseline +/- up to 7 days)	Follow up visit (135 days +/- 7 days from baseline)	End of study visit (180 days from baseline +/- up to 7 days)
Informed consent		X					
Demographics		X					
Medical History		X					
Current / Concomitant medication		X	X	X	X	X	X
General physical examination		X	X	X	X	X	X
Vital signs		X	X	X	X	X	X
Hemoglobin (Hb) assessment using Pronto		X	X	X	X	X	X
Anthropometric measurements (height, weight and BMI)		X					
Inclusion / Exclusion criteria evaluation		X					
Subject Eligibility		X					
Randomization		~~X~~	X				
Continued Eligibility			X	X	X	X	X
7-day diary for assessment of dietary intake	Diary and weighing scale distribution	X				X@	
	Diary and weighing scale collection		X				X
Pregnancy Test		X	X	X	X	X	X
Blood Sample collection for analysis of ~~serum~~-CTX-1, NTX-1, P1NP, BSAP, total OC, cOC, uOC, PTH, 25 (OH) D, Ca, P, total ALP, IGF-1, Zn, Se, B6, B12 and Folic acid			X		X		X
URINE Sample collection for analysis of urinary calcium, creatinine and CTX-1			X		X		X
Product Administration and dispensing			X	X	X	X	X$
Collection of returned packs				X	X	X	X
Adverse Events monitoring			X	X	X	X	X
Study Conclusion/ Medical Sign-off							X

(*Diary will be collected on ~~first monitoring~~ SUBSEQUENT visit after distribution, #product compliance assessment will be done on ~~visits~~ EVERY VISIT ~~monitoring~~ AFTER VISIT 2, @ Telephonic follow up will be done 8-10 days prior to visit-6 to remind filling of food diary for last seven days of intervention/7 days prior to visit -6, $To be consumed until last evening before visit-6 not actually on the day because we are drawing last blood sample fasting and after that it is not needed)

PROTOCOL SYNOPSIS FOR STUDY 207192

Brief Summary

Change in lifestyle, dietary habits and environment is known to have significant effect on women's physiology and bones are not spared from the impact. In postmenopausal women, the prevalence of low bone mineral density (BMD), its association to fracture risk and the efficacy of pharmacologic therapy to reduce the risk of fractures are well established. The World Health Organisation criteria for diagnosing osteopenia (BMD score between 1 and 2.5 Standard Deviation (SD) below the young adult mean i.e. between −1 to −2.5 SD) and osteoporosis (BMD score of 2.5 SD or more below the young adult mean i.e. -2.5 SD or lower) are also based on epidemiological data obtained in post- menopausal women and the treatment guidelines are also targeted for postmenopausal women. This is mainly because bone mass and bone turnover are generally thought to remain stable from the end of puberty to menopause. However, several cross-sectional & longitudinal studies have reported that bone mineral density (BMD) and bone turnover markers change between the 2nd to the 4th decade of life. In Indian females, bone mineral density reaches a peak at age 28 years for femur, at 29 years for forearm and at the age of 20 years for lumbar spine. Further, it is reported that Indians have significantly lower BMD & attain lower Peak Bone mass (PBM) than their North American counterparts (Mukherjee et al., 2010). Post attainment of PBM, significant loss in bone mass occurs as evidenced by very high prevalence of osteopenia and osteoporosis in premenopausal indian women (Mithal et al., 2009, Mithal et al., 2014, Agarwal & Verma 2013, Silvanus et al., 2012, Kadam et al., 2010 Desai et al., 2007).

In the 2009 International Osteoporosis Foundation (IOF) Asian Audit, expert groups estimated that the number of osteoporosis patients in India was approximately 26 million in 2003, with projections indicating that this would rise to 36 million patients by 2013(Mithal et al.,2009) . In 2013, sources estimate that 50 million people in India are either osteoporotic or have low bone mass (Mithal et al., 2014)

Agarwal and Verma (2013) reported 48% prevalence of osteopenia & 13% of osteoporosis among urban Indians women above the age of 35 years. A cross sectional study comparing BMD and its variables in premenopausal (age: 32.46±7.8 years) and postmenopausal women (age : 51.74±7.1 years) showed that significant number of women (43.54%) had osteopenia during premenopausal period while 9.6% of the premenopausal women also had osteoporosis (Mittal et al., 2011).

Reasons ascribed for lower PBM and BMD in Indians other than genetic differences is primarily nutritional deficiencies, in particular Calcium and Vitamin D deficiency during growth years, adult hood and older years. For e.g. the reported median dietary calcium intakes for Indian adults is between 300-350mg/day (Mithal et al., 2009; Harinarayan et al., 2007). Considering that Adult RDA of Calcium for Indian's is 600mg, there exists a deficit of 250-300mg/day. Similarly,

Vitamin D deficiency has been shown unequivocally across all ages throughout India, despite abundant sunshine. Vitamin D deficiency during childhood and adolescence decreases peak bone mass in adults and may increase the risk of developing osteoporosis (Shivane et al., 2012). Studies indicate approximately 80-90% of hip fracture patients in India are vitamin D deficient (Dhanwal et al., 2013 ; Khadgawat et al., 2013). The high rate of vitamin D deficiency may be due to several causes such as low sun exposure, inadequate dietary vitamin D intake, lack of food fortification, pigmented skin, environmental pollution and clothing habbits (Mithal et al., 2009). Studies have shown that desirable serum levels of Vitamin D (25(OH)D) is between 25 to 45 ng/mL (50 to 100 nmol/L) based on beneficial effects on bone health such as prevention of fall and fracture risk (Fuleihan et al., 2015) . Considering that more than 80% of urban Indians (across age groups) have serum 25(OH) D levels below 20 ng/mL (Mithal et al., 2014) and that for every 100 IU (2.5 mcg) additional Vitamin D each day over several months, serum 25(OH)D can be expected to rise by about 1 ng/mL (Heaney, 2008), significant amount of Vitamin D are required to be added from the supplements to attain adequate serum levels.

Hence, there exists a need for appropriate supplements containing Calcium, Vitamin D and other osteogenic nutrients to improve bone health across life-stages. Premenopausal women is an important target group as they may still have the opportunity to increase their peak bone mass. There is evidence that physically active 20 to 50 year old women with a good lifetime calcium intake can increase their peak bone mass (Halioua & Anderson et al., 1989). Further, supplementation during premenopausal years helps to maintain bone mass such that women enter menopause (period of accelerated bone loss due to estrogen deficiency) with higher bone stores. Data in Indian population have revealed that average age for menopause in India is 47 years and that significant increase in Bone turnover is observed after the age of 40 years due to hormonal changes (Desai et al., 2007). Once the hormonal changes set-in, bone metabolism becomes less amenable to nutritional interventions. Hence, nutritional supplements to improve bone mass in second and third decades of life are particularly beneficial to help attain adequate peak bone mass and maintain the same through the premenopausal years. Such strategies would go a long way to reduce the morbidity of osteoporosis later in life. It is reported that a 10% increase in PBM corresponds to a gain of one standard deviation in bone mineral density in adulthood & osteoporotic fracture risk may be reduced by up to 50% (Marshall et al., 1996)

GSK India consumer healthcare has developed a customised product designed to improve bone health in Indian Women. It is rich in good quality protein and several osteogenic nutrients like Calcium, Vitamin D, Vitamin K, Magnesium, Selenium, Vitamin C etc. Greater details on the product are discussed in section 3. The objective of this study is to test the efficacy of this supplement on bone health by validated methods.

Objectives and Endpoints

Objectives	Endpoints
Primary	
To compare the changes in Bone Resorption marker: serum C-telopeptide of type 1 collagen (s-CTX-1) at endline 6 months from baseline in test and control groups	Change from baseline in s-CTX-1 at 6 months
To compare the changes in the ratio of carboxylated (c-OC) to under-carboxylated Osteocalcin (uc-OC) at endline 6 months from baseline in test and control groups	Change from baseline in the ratio of c-OC to uc-OC at 6 months
Secondary	
To compare the changes in Bone Resorption marker: serum C-telopeptide of type 1 collagen (s-CTX-1) at midline 3 months from baseline in test and control groups	Change from baseline in s-CTX-1 at 3 months
To compare the changes in the ratio of carboxylated (c-OC) to under-carboxylated Osteocalcin (uc-OC) at midline 3 months from baseline in test and control groups	Change from baseline in the ratio of c-OC to uc-OC at 3 months
To compare the changes in Bone Resorption marker: urinary CTX-1 at midline 3 months and endline 6 months from baseline in test and control groups	Change from baseline in Urinary CTX-1 at 3 and 6 months
To compare the changes in Bone Resorption marker: serum N-terminal telopeptide of type 1 collagen (s-NTX-1) at midline 3 months and endline 6 months from baseline in test and control groups	Change from baseline in s-NTX-1 at 3 and 6 months
To compare changes in Bone formation marker serum procollagen type 1 N-terminal propeptide (s-P1NP), bone specific alkaline	Change from baseline in s-P1NP, BSAP at 3 and 6 months

phosphatase (BSAP) at baseline, at midline 3 months and endline 6 months in test and control groups	
To compare Calcium Status using s-PTH & Urinary Calcium, Serum Ca, P and total alkaline phosphatase (ALP) at midline 3 months and endline 6 months to see change in level from baseline in both the groups	Change from baseline in s-PTH & Urinary Calcium, Serum Ca, P and total alkaline phosphatase (ALP) at 3 and 6 months
To compare the changes in Vitamin D status using serum 25-hydroxycholecalciferol (25 OH D3) at midline 3 months and endline 6 months from baseline in test and control groups	Change from baseline in 25-hydroxycholecalciferol (25 OH D3) at 3 and 6 months
To compare the changes in Micronutrient Profile status using serum Se, ~~Zn~~, Folic acid, ~~B6 and~~ B12 **AND PLASMA Zn AND B6** status at midline 3 months and endline 6 months from baseline in test and control groups	Change from baseline in Serum Se, ~~Zn~~, Folic acid, ~~B6~~ and B12 **AND PLASMA Zn AND B6** status at 3 and 6 months
Exploratory	
To compare the changes in Growth Markers IGF-1 at midline 3 months and endline 6 months from baseline in test and control groups	Change from baseline in IGF-1 at 3 and 6 months

Study Design

Overall Design
This is a double blind, single-centre, randomized-controlled trial testing the effect of fortified beverage on bone turnover markers as compared to placebo control in 25-45 yrs old premenopausal women. Group 1:- Protein rich beverage powder fortified with MMN Group 2:- Low protein non-fortified iso-caloric beverage powder

Statistical Methods

The co-primary efficacy variables of the study are the change from baseline in bone resorption marker (s-CTX-1) after 6 months and the change from baseline in the ratio of carboxylated to under carboxylated osteaocalcin (CoC/uCoc) after 6 months.

Each of these co-primary efficacy variables will be analyzed using the analysis of covariance (ANCOVA). The ANCOVA will have treatment as fixed effect and the corresponding baseline as covariate. Adjusted means, 95% confidence intervals, within treatment p-values for each treatment group, treatment group difference, 95% confidence interval of the difference and the between-treatment p-values based on the statistical model described above will also be presented. Statistical tests to compare treatments will be two-sided and will employ a level of significance of $\alpha = 0.025$.

The setting of the significance level of 0.025 for these 2 co-primary endpoints will ensure that the overall significance level for the primary endpoint is less than or equal to 0.05.

Assumptions of normality and homogeneity of variances will be evaluated. If violations are observed then suitable data transformations will be tried to achieve the assumptions. If suitable transformations cannot be found, non-parametric analyses will be performed.

The secondary and exploratory analyses will be performed in a similar way as the primary analyses, though there will be no adjustments made for multiplicity.

1. INTRODUCTION

It is well documented that in the field of nutrition, valuable information could be obtained from randomised controlled trials that have Bone turnover markers (BTMs) as endpoints to demonstrate changes in bone metabolism and that such data could be used as scientific support for claims on foods or nutrients that influence bone metabolism (Bonjour et al., 2014). BTM are products released into the bloodstream during bone formation and resorption and can be used as surrogate markers of bone turnover. These markers include bone-specific alkaline phosphatase (ALP), PINP (N-terminal propeptide of type I procollagen), osteocalcin (OC), urinary levels of pyridinolines and deoxypyridinolines, serum levels of type I collagen telopeptides ; N-terminal cross-linking telopeptide of type I collagen, NTX-I and C-terminal cross-linking telopeptide of type I collagen, CTX-I etc. These markers are sensitive and respond quickly to nutrition or drug interventions. In short-term studies, the measurement of biochemical markers to assess bone turnover offers an accepted means of determining the effect of a treatment or dietary supplement on bone metabolism before any significant change in actual bone density can be detected (et al., 2000). Several studies have now shown that increased levels of bone resorption markers are associated with an increased risk of fracture suggesting that increased bone resorption leads to

micro-architectural deterioration of bone tissue due to perforation of the trabeculae (Garnero et al., 1996). In South East Asian premenopausal women, fortified milk with Calcium, vitamin D or K1 have shown to rapidly reduce bone resorption and bone turnover markers within weeks of starting supplementation (Kruger et al., 2006). This emphasises the importance of bone turnover marker response in monitoring treatment effects in the short- to medium-term. As BTMs can pick changes quite early, they are a very helpful tool to modulate the treatment/intervention in subjects who do not show the expected response, if necessary. On the other hand, BMD changes may take 18 months to become significant; thus, it takes very long to detect treatment failure using the DEXA. Further, in clinical trials, biochemical markers provide a superior signal-to-noise ratio, and therefore, these may be used to shorten duration of trials and to decrease study cohorts in size. In a study of treatment of postmenopausal osteoporosis with alendroante, it was reported that the signal to noise ratio for the bone turnover marker NTX was 4.9 whereas the ratio for lumbar spine BMD was only 1.3 (after 6 months of treatment) (Machado, 1999).

Insights on relevance of BTM assessment in young premenopausal women is provided by two prospective cohort studies which have shown that BTMs assessment in premenopausal women can predict fracture risk later in life (during postmenopausal years) (i) Cauley et al., (2012) in a 7 year study showed that that higher urinary NTX-I excretion measured before menopause and across menopausal transition is associated with a higher risk of fracture. The study comprised of 2,305 women (42 to 52 years) who were either premenopausal or early perimenopausal at baseline. Serum OC and urinary NTX-I were measured at baseline. NTX-I was measured at each annual follow-up. Women who experienced fractures (n = 184) had about a 10% higher baseline median NTX-I (P = 0.001), but there was no difference in OC. Further, the women with a baseline NTX-I greater than the median had a 45% higher risk of fracture, multivariable-adjusted (HR, 1.46; 95% CI, 1.05-2.26). (ii) Japanese Population-based Osteoporosis Study reported that biochemical markers of bone turnover predicted bone loss in perimenopausal women but not in postmenopausal women. A cohort of 1,283 women aged 15–79 years was selected randomly from the inhabitants of three areas in Japan and followed for 6 years. The annual changes in bone density at the spine, total hip, and distal radius and changes in bone turnover markers including serum OC and bone-specific alkaline phosphatase (bone ALP), free and total tDPD , urinary CTX-I were measured. Perimenopausal women aged 45 years or older with elevated levels of OC, bone ALP, CTX-I, or tDPD showed significantly greater bone loss at most skeletal sites during the follow-up period than those with lower levels, after adjustment for the effects of age, height, weight, dietary calcium intake, regular exercise, and current smoking (Iki et al., 2006).

International Osteoporosis foundation (IOF) recommends the use of s-P1NP as the reference bone formation marker and CTX-1 as the reference bone resorption marker to assess changes in bone metabolism post intervention (Vasikaran et al., 2011). GREES panel (Group for respect of ethics and excellence in Science; one of the most prominent scientific groups working in perspective of improving patient well-being) also supports IOF recommendations to measure changes in BTMs to support claims on food products for bone health (Bruyere et al., 2012)

Hence, for the present study, these two reference Bone turnover markers CTX and P1NP recommended by IOF will be utilised to assess the impact of Women's Horlicks (a customised nutritional supplement for bone health rich in osteogenic nutrients) in Indian health pre-menopausal women the age group 25-45 years(inclusive), following six months supplementation. Including women between 25-45 years provides an opportunity to test the efficacy of the supplement in improving Peak bone mass acquisition as bone accrual continues during the age period 20-30 years. Hence, we propose to include a sub-analysis for 'attainment of peak bone mass' and 'attained peak bone mass'. The period of attainment of peak bone mass includes bone modelling as well as remodelling and the process is dependent on IGF-I. Nutrients such as protein and zinc have been shown to increase IGF-I and so could increase bone formation (PINP). Further, Women are prone to vitamin D deficiency during this period and so a beneficial effect of vitamin D and calcium supplements may show itself through lower PTH and bone markers (CTX, PINP). The period of attained peak bone mass includes mostly bone remodelling and so a benefit would be best shown with a decrease in bone resorption (CTX). Hence, CTX is proposed as the primary objective for the study, the secondary being, changes in PINP and other bone markers.

The study will be funded by GlaxoSmithKline Consumer Healthcare (GSKCH) and will be run in India as a randomised double blind, controlled study.

2. OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary	
To compare the changes in Bone Resorption marker: serum C-telopeptide of type 1 collagen (s-CTX-1) at endline 6 months from baseline in test and control groups	Change from baseline in s-CTX-1 at 6 months
To compare the changes in the ratio of carboxylated (c-OC) to under-carboxylated Osteocalcin (uc-OC) at endline 6 months from baseline in test and control groups	Change from baseline in the ratio of c-OC to uc-OC at 6 months
Secondary	
To compare the changes in Bone Resorption marker: serum C-telopeptide of type 1 collagen (s-CTX-1) at midline 3 months from baseline in test and control groups	Change from baseline in s-CTX-1 at 3 months

To compare the changes in the ratio of carboxylated (c-OC) to under-carboxylated Osteocalcin (uc-OC) at midline 3 months from baseline in test and control groups	Change from baseline in the ratio of c-OC to uc-OC at 3 months
To compare the changes in Bone Resorption marker: urinary CTX-1 at midline 3 months and endline 6 months from baseline in test and control groups	Change from baseline in Urinary CTX-1 at 3 and 6 months
To compare the changes in Bone Resorption marker: serum N-terminal telopeptide of type 1 collagen (s-NTX-1) at midline 3 months and endline 6 months from baseline in test and control groups	Change from baseline in s-NTX-1 at 3 and 6 months
To compare changes in Bone formation marker serum procollagen type 1 N-terminal propeptide (s-P1NP), bone specific alkaline phosphatase (BSAP) at baseline, at midline 3 months and endline 6 months in test and control groups	Change from baseline in s-P1NP, BSAP at 3 and 6 months
To compare Calcium Status using s-PTH & Urinary Calcium, Serum Ca, P and total alkaline phosphatase (ALP) at midline 3 months and endline 6 months to see change in level from baseline in both the groups	Change from baseline in s-PTH & Urinary Calcium, Serum Ca, P and total alkaline phosphatase (ALP) at 3 and 6 months
To compare the changes in Vitamin D status using serum 25-hydroxycholecalciferol (25 OH D3) at midline 3 months and endline 6 months from baseline in test and control groups	Change from baseline in 25-hydroxycholecalciferol (25 OH D3) at 3 and 6 months
To compare the changes in Micronutrient Profile status using serum Se, ~~Zn~~, Folic acid, ~~B6~~ and B12 status **AND PLASMA Zn AND B6** at midline 3 months and endline 6 months from baseline in test and control groups	Change from baseline in Serum Se, ~~Zn~~, Folic acid, ~~B6~~ and B12 **AND PLASMA Zn AND B6** status at 3 and 6 months
Exploratory	

To compare the changes in Growth Markers IGF-1 at midline 3 months and endline 6 months from baseline in test and control groups	Change from baseline in IGF-1 at 3 and 6 months

3. STUDY PLAN

3.1. Study Design

Overall Design
This is a double-blind, single-centre, randomized-controlled trial testing the effect of fortified beverage on bone turnover markers as compared to placebo control in 25-45 yrs old premenopausal women. Group 1:- Protein rich beverage powder fortified with MMN Group 2:- Low protein non-fortified iso-caloric beverage powder
Visit 1 - Screening Visit
The following assessments will be conducted ~~in the written order~~ Informed consent Demographics Medical history Current/concomitant medication General physical examination Vital signs Hb assessment using Pronto Anthropometric measurements (height, weight and BMI) Inclusion/ exclusion criteria evaluation Subject eligibility

~~**Randomisation**~~ Pregnancy test 7-day Food intake diary hand over to participant
Visit 2 - Baseline Visit (+7 days to 10 days post screening)
The following assessments will be conducted ~~**in the written order**~~ Current and concomitant medication Continued eligibility Pregnancy Test **RANDOMISATION** 7-day diary collection for assessment of dietary intake of calcium, protein & other key micronutrients. **GENERAL PHYSICAL EXAMINATION** **VITAL SIGNS** **HB ASSESSMENT USING PRONTO** ~~**Pregnancy Test**~~ Sample collection for analysis of serum CTX-1, NTX-1, P1NP, BSAP, total OC, cOC, uOC, PTH, 25 (OH) D, Ca, P, total ALP, IGF-1, ~~Zn~~, Se, ~~vitamin B6~~, vitamin B12 and Folic acid, **PLASMA FOR Zn AND VITAMIN B6** Sample collection for analysis of urinary calcium, creatinine and CTX-1 Product administration and dispensation Adverse events monitoring
Visit 3 – Follow-up visit (45 days +/- 7 days from visit 2)
The following assessments will be conducted ~~**in the order written:**~~ Current and concomitant medication

Continued eligibility Pregnancy test **GENERAL PHYSICAL EXAMINATION** **VITAL SIGNS** **HB ASSESSMENT USING PRONTO** Product administration and dispensation Collection of return sachet (empty+ unconsumed) Adverse events monitoring
Visit 4 – Midline visit (90 days +/- 7 days from baseline)
The following assessments will be conducted ~~in the order written:~~ Current and concomitant medication Continued eligibility Pregnancy test **GENERAL PHYSICAL EXAMINATION** **VITAL SIGNS** **HB ASSESSMENT USING PRONTO** Sample collection for analysis of serum CTX-1, NTX-1, P1NP, BSAP, total OC, cOC, uOC, PTH, 25 (OH) D, Ca, P, total ALP, IGF-1, ~~Zn~~, Se, ~~vitamin B6~~, vitamin B12 and Folic acid, **PLASMA FOR Zn AND VITAMIN B6** Sample collection for analysis of urinary calcium, creatinine and CTX-1 Product administration and dispensation Collection of return sachet (empty+ unconsumed) Adverse events monitoring
Visit 5 – Follow-up visit (135 days +/- 7 days from baseline)

The following assessments will be conducted ~~in the order written~~: Current and concomitant medication Continued eligibility Pregnancy test **GENERAL PHYSICAL EXAMINATION** **VITAL SIGNS** **HB ASSESSMENT USING PRONTO** Product administration and dispensation Collection of return sachet (empty+ unconsumed) Adverse events monitoring 7-day Food intake diary hand over to participant (there will be telephonic reminder 8-10 prior to visit 6 to start filling diary according to instruction)
Visit 6 – End of study (180 days/LSLV +/- up to 7 days from baseline)
PRODUCT ADMINISTRATION (TO BE CONSUMED UNTIL LAST EVENING BEFORE VISIT-6 NOT ACTUALLY ON THE DAY BECAUSE WE ARE DRAWING LAST BLOOD SAMPLE FASTING AND AFTER THAT IT IS NOT NEEDED) The following assessments will be conducted ~~in the order written~~: Current and concomitant medication ~~General physical examination~~ ~~Vital signs~~ Continued eligibility **PREGNANCY TEST** **GENERAL PHYSICAL EXAMINATION** **VITAL SIGNS**

HB ASSESSMENT USING PRONTO

Collection of return sachet (empty+ unconsumed)

7-day diary collection for assessment of dietary intake of calcium, protein & other key micronutrients

~~**Pregnancy Test**~~

Sample collection for analysis of serum CTX-1, NTX-1, P1NP, BSAP, total OC, cOC, uOC, PTH, 25 (OH) D, Ca, P, total ALP, IGF-1, ~~Zn~~, Se, ~~vitamin B6~~, vitamin B12 and Folic acid, **PLASMA FOR Zn AND VITAMIN B6**

Sample collection for analysis of urinary calcium, creatinine and CTX-1

Adverse events monitoring

Study conclusion/Medical sign off

3.2. Subject Restrictions

Lifestyle/ Dietary
Lifestyle : The following lifestyle restriction applies during the study intervention period; from baseline/start of product administration to end of study visit: Participant must agree to abstain from use of any health food drinks/beverages and any supplements, including herbal supplements and nutritional supplements, including health food supplements. Participants should maintain their regular activities but must not include any special physical activity.
Medications and Treatments
There are no restrictions with regards to medication use and medical treatment. However use of drugs such as glucocorticoids, anti-convulsants, selective estrogen receptor modulators (e.g. raloxifene etc), bisphosphonates (e.g. alendronate etc) (for more than a 7 days) or any other medication which can impact overall bone health, during the course of study post screening, will be considered a protocol violation and will result in the removal of the subject from the efficacy analyses.

3.3. Type and Planned Number of Subjects

Approximately 135 participants will be screened to randomise approximately 108 participants (54 per treatment arm) in order to obtain atleast 88 participants completing the study.

3.4. Study Design and Dose Justification

This is a double blind, two-arm, parallel group, stratified (for age bands), randomized, controlled, study in women 25-45 yrs, inclusive. The study centre will aim to recruit a target ratio of 50% 25-35 years age and 50% 35-45 years with a minimum of 40% of either age band.

Randomization of the subjects will ensure that bias is minimized by distributing the characteristics of subjects that may influence outcome randomly between treatment groups, so that any difference in outcome can be explained only by treatment. The control group will allow the determination of whether improvement in outcomes, if any, is related to intervention product. To ensure compliance, the consumption of the study product will be monitored by the study staff.

The study will be double blinded with respect to the subject and the study staff (those involved in assessment) to ensure there is no bias in the assessments. The subjects in this study will be randomly assigned to either the test treatment or the control treatment.

4. SELECTION OF STUDY POPULATION AND WITHDRAWAL CRITERIA

Females, aged between 25 to 45 years inclusive will be invited to participate in this study.

Specific information regarding warnings, precautions, contraindications, adverse events, and other pertinent information on the GSK investigational product or other study treatment that may impact subject eligibility is provided in the Safety Statement.

Deviations from inclusion and exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

4.1. Inclusion Criteria

A subject will be eligible for inclusion in this study only if all of the following criteria apply:

1. CONSENT
Demonstrates understanding of the study procedures, restrictions and willingness to participate as evidenced by voluntary written informed consent and has received a signed and dated copy of the informed consent form.
2. AGE
Aged between 25 and 45 years inclusive.

3. GENDER
Subject is female.

4. GENERAL HEALTH
Good general and mental health with, in the opinion of the investigator or medically qualified designee no clinically significant and relevant abnormalities in medical history or upon physical examination.

5. WEIGHT/ BMI/ Z SCORES
BMI between 18.0-30 (kg/m^2) inclusive.

6. COMPLIANCE
Women who understand, willing, able and likely to comply with all study procedures and restrictions.

4.2. Exclusion Criteria

A subject will not be eligible for inclusion in this study if any of the following criteria apply:

1. PREGNANCY

Women who are known to be pregnant or who are intending to become pregnant over the duration of the study. OR Women who have a positive urine pregnancy test.

2. MENOPAUSE
Women who have attained physiological menopause defined as those who have not had a menstrual period for consecutive 12 months.

3. BREAST-FEEDING
Women who are breast-feeding

4. CONCURRENT MEDICATION/ MEDICAL HISTORY
A. Current (within 14 days of the start of the study) or regular use of any prescription, over-the-counter (OTC), vitamin supplements herbal medicine unless the medication has been approved by the study physician. B. Treatment with bisphosphonates (any dose within the previous 2 years) or other medications known to affect bone (within the previous 6 months), C. History of metabolic bone disease D. Any hormonal disorders or disturbances E. Bone fracture in last 12 months

5. ALLERGY/ INTOLERANCE
A. Known or suspected intolerance or hypersensitivity to the study materials (or closely related compounds) or any of their stated ingredients.

B. Subject is lactose intolerant.

6. CLINICAL STUDY/ EXPERMENTAL PRODUCT

A. Participation in another clinical study (including cosmetic studies) or receipt of an investigational drug within 30 days of the screening visit.

B. Previous participation in this study.

7. SUBSTANCE ABUSE

A. Recent history (within the last 1 year) of alcohol or other substance abuse.

B. More than 2 Units of alcohol per day

C. Smokers

8. FOOD/ BEVERAGE

A. Currently taking any other health food drinks/beverages or supplements (including nutritional supplements e.g. multivitamins and/or herbal supplements e.g. ginkgo) or has been on supplements within a month prior to study start.

B. Women who used medication known to influence bone mass and the use of calcium, vitamin D, and multivitamin supplements on a regular basis were stopped 2 months before the onset of the trial.

9. PERSONNEL

An employee of the sponsor or the study site or members of their immediate family.

10. DIAGNOSTIC ASSESSMENTS AND OTHER CRITERIA

A. Ongoing conditions known to cause abnormalities of calcium metabolism or skeletal health, malabsorption syndromes (such as coeliac or Crohn's disease), hyperthyroidism,

hyperparathyroidism, hypo- or hypercalcaemia, osteomalacia, Paget's disease, diabetes,

B. Fracture in the past 12 months,

C. Known chronic kidney disease, alcoholism,

D. Obesity women (BMI>30) and Thinness i.e. BMI<18 kg/m2

E. Severely anemic (Hb<8 g/dl ~~mg%~~)

F. Undertaking excessive exercise (>2 strenuous* exercise sessions per week)

G. Contraceptive injections within the previous year.

H. Known histories of surgeries such as bilateral oophrectomy (surgical removal of ovaries)

I. Diagnosed hypogondal states such as Turner syndrome, Klinfelter syndrome, Kallman syndrome, anorexia nervosa, hypothalamic amenorrhea or hyperprolactinemia

J. Hemotological disorders e.g. Hemophilia, Leukemia and lymphomas monoclonal multiple myeloma, sickle cell disease, Thalassemia etc.

K. Rheumatological and autoimmune disorders such as ankylosing spondylitis, rheumatoid arthritis, systemic lupus etc.

L. Miscellaneous conditions and diseases such as HIV/AIDS, alcoholism, amyloidosis, chronic metabolic acidosis, COPD, congestive heart failure, depression etc.

* Resistance training, running and athletic activities

4.3. Screening/ Baseline Failures

Screen failures are defined as subjects who consent to participate in the study but are never subsequently randomized. In order to ensure transparent reporting of screen failure subjects, a minimal set of screen failure information is required including Demography, Screen Failure details, Eligibility Criteria, and any Serious Adverse Events. Re-screening of subjects will not be allowed in this study.

4.4. Withdrawal/ Stopping Criteria

A subject may withdraw from the study at any time at his/her own request, or may be withdrawn at any time at the discretion of the investigator for safety, behavioural or administrative reasons. If a subject withdraws or is withdrawn from the study, all human biological samples collected before they left will be analyzed and reported unless the subject requests otherwise. A subject may request for their human biological samples to be destroyed. In these cases, the investigator

must document this in the site study records and the samples should not be used for any further research.

If the reason for removal of a subject from the study is an AE or an abnormal laboratory test result, the principal specific event or test will be recorded on the electronic case report form (CRF). If a subject is withdrawn from the study because of a product limiting AE, thorough efforts should be clearly made to document the outcome. Any AEs ongoing at the final visit will be followed up until resolved, the condition stabilizes, is otherwise explained, or the subject is lost to follow-up.

The following actions must be taken in relation to a subject who fails to attend the clinic for a required study visit:

The site must attempt to contact the subject and re-schedule the missed visit as soon as possible.

The site must counsel the subject on the importance of maintaining the assigned visit schedule and ascertain whether or not the subject wishes to and/or should continue in the study.

In cases where the subject is deemed 'lost to follow up', the investigator or designee must make every effort to regain contact with the subject (where possible, at least 2 telephone calls). The contact attempt should be documented in the subject's record.

Should the subject continue to be unreachable, only then will he/she be considered to have withdrawn from the study with a primary reason of "Lost to Follow-up".

4.5. Subject Replacement

Subjects who withdraw from the study post-randomization will not be replaced.

4.6. Subject and Study Completion

A completed subject is one who has completed all phases of the study including the follow-up visit.

The end of the study is defined as the date of the last subject's last visit.

5. PRODUCT INFORMATION

5.1. Study Product

The following study products will be supplied by the Clinical Supplies Department, GSKCH:

	Test Product 1	Reference Product 1
Product Name	Cereal based fortified Beverage (Proprietary food)	Low protein non fortified isocaloric beverage
Product Formulation Code (MFC)	CCI	CCI
Dose	60 grams daily in two divided doses	60 grams daily in two divided doses
Route of Administration	Oral	Oral
Dosing Instructions	30 grams powder made up in 200 mL water, administered twice daily	30 grams powder made up in 200 mL water administered twice daily

Other items to be supplied by the Clinical Supplies Department, GSKCH:

Name of Item	Purpose
Graduated tumblers	Dose preparation
Stirrers	Dose preparation

5.2. Dose Schedule

Participants will be administered two doses of the drink (approx 200 mL each) daily for 6 months. Each day one dose will be administered first in the morning and the second dose will be administered preferably in evening

5.3. Dose Administration

The graduated tumbler will be filled with water up to the 200 mL mark. The entire contents of one sachet will be gradually emptied in the water filled tumbler with intermittent stirring to avoid formation of lumps. The reconstituted test product will be consumed by the participants immediately orally.

5.4. Dose Modification

No dose modification is permitted in this study. Any variation from the dosing regimen will be reported to the study site personnel and details will be entered on the test product administration log and then entered into the CRF.

5.5. Product Compliance

Random home visits will be made by site staff to ensure compliance of product consumption at home. Details of missed product consumption will be recorded in compliance register later will be entered in the CRF.

Participant will need to enter details in the **SUBJECT DIARY CARD FOR DOSE COMPLIANCE** ~~Product Compliance Report~~. Site staff will dispense as many blank **SUBJECT DIARY CARDS** ~~reports~~ as appropriate for recording details. Each **SUBJECT DIARY CARD** ~~report~~ will be numbered ~~in an ascending order (for each participant) and records should be maintained to track the total number of reports dispensed and collected~~. Participant will be considered as non-compliant if the participant consumes less than 80% of the total amount of recommended (for the entire study duration) dose.

5.6. Precautions

No special precautions are necessary provided the test product is used in accordance with the protocol and the usage instructions.

5.7. Overdose

An overdose is a deliberate or inadvertent administration of a product at a dose higher than specified in the protocol.

Overdose is not likely to occur in this study. Limited quantities of the product will be supplied, and closely monitored by the site for each subject.

Overdose per se is not an AE. However, any clinical sequelae of an overdose should be reported as an AE (and serious adverse event (SAE), if appropriate). For reporting, follow the AE and SAE reporting instructions.

5.8. Rescue Therapy

No rescue therapy is required in this study.

5.9. Product Assignment

Subjects will be assigned to study product in accordance with the randomization schedule generated by the Biostatistics Department, GSKCH, prior to the start of the study, using validated internal software.

5.9.1 Randomization

A unique screening number will identify each subject screened for study participation. Screening numbers will be assigned in ascending numerical order as each subject signs their consent form. Subjects who meet all inclusion and exclusion criteria will be assigned to one of the two study treatments according to a randomisation schedule provided by the Biostatistics Department, GSKCH. The study products will be labelled with randomisation numbers according to this randomisation schedule. Subjects will be randomised in an ascending numerical order as they qualify for study entry stratified by their age group.

The strata are as follows:

Stratum 1: Age ≥ 25 years; age <35 years

Stratum 2: Age ≥ 35 years; age ≤ 45 years.

The randomisation schedule with treatment decodes will be provided to the site in a sealed envelope and will be available for emergency use only. All randomisation numbers will be masked with scratch-off panels. Only the panels required for emergency subject unblinding should be removed.

5.9.2 Blinding

The study is a double-blind trial; neither the sponsor, sponsor's representative, investigator/site staff, nor the participating subject will know which treatment an individual is randomised.

5.9.3 Code Breaks

The blind must only be broken in an emergency where it is essential to know which product a subject received in order to give the appropriate medical care. Wherever possible the Investigator (or designee) must contact the Sponsor prior to breaking the blind. The investigator must document the reason for breaking the code and sign and date the appropriate document.

The randomization schedule must be returned to GSKCH at the end of the study.

5.10. Packaging and Labelling

The contents of the label will be in accordance with all applicable regulatory requirements and will be the responsibility of the Clinical Supplies Department, GSKCH.

The test and reference products will be supplied in plain packaging with study labels affixed to each sample. The study centre will be supplied a labelled monthly subject carton for each participant containing sufficient sachets for the period of use.

Care should be taken with the supplied products and their labels so that they are maintained in good condition. It is important that all labels remain intact and legible for the duration of the study. Subjects should be instructed to not remove or deface any part of the study label.

5.10.1. Accountability of Product

All products supplied are for use only in this clinical study and should not be used for any other purpose.

The investigator or designee will maintain a full record of study product accountability. A Product Dispensing Log must be kept current and will contain the following information:

The identification of the subject to whom the study product was dispensed.

The date and quantity of the study product dispensed to the subject.

The date and quantity of the study product returned by the subject (if applicable).

The inventory must be available for inspection by the study monitor during the study. At the end of the study, study product supplies will be verified by the monitor. Study product supplies will then be either collected by the study monitor or returned by the investigator or designee to the GSKCH Clinical Supplies Department or designated vendor.

5.10.2. Storage of Product

Study product supplies must be stored in compliance with the label requirements in a secure place with limited or controlled access.

6. STUDY ASSESSMENTS AND PROCEDURES

This section lists the procedures and parameters of each planned study assessment. The exact timing of each assessment is listed in the Schedule of Events section.

Adherence to the study design requirements, including all assessments and procedures are essential and required for study conduct.

6.1. Telephone Screening

Prior to the screening visit, telephone screening of interested subjects will be conducted using a telephone script. This will be conducted by the site recruitment staff or designee.

6.2. Informed Consent

The investigator, or designee, must obtain written (signed and dated by the subject) informed consent from each subject participating in this study after adequate explanation of the aims, methods, objectives, and potential hazards of the study.

GSK and the investigator must agree on the process followed to ensure that the appropriate measures are taken to establish legally acceptable representative or impartial witness is compliant with local regulatory or corporate expectations prior to study start.

The investigator, or designee, must also explain to the subjects that they are completely free to refuse to enter the study or to withdraw from it at any time. Appropriate forms for documenting a written consent will be provided by the investigator or by GSKCH. The investigator, or designee, should sign and date the consent form to confirm that the consent process was completed correctly. The subject will be provided with a copy of their signed and dated consent form and any other written information which they should be instructed to retain.

If, during a subject's participation in the study, any new information becomes available that may affect the subject's willingness to participate in the study, each ongoing subject should receive a copy of this new information and be re-consented into the study. Subjects should be provided with a copy of the signed and dated amended consent form. The date of consent will be recorded on the CRF.

6.3. Demographics

The following demographic parameters will be captured by the Investigator or designee and recorded on the CRF: year of birth, gender and race.

6.4. Medical History and Concomitant Medication

Medical history will be assessed as related to the inclusion/exclusion criteria by the Investigator or medically qualified designee. Details of any relevant medical or surgical history including allergies or drug sensitivity will be recorded on the CRF. Any concomitant therapy taken in the prior to the Screening Visit and throughout the study will also be recorded.

6.5. General Physical Examination

The Investigator or their designee will perform a physical examination which should include assessment of the following body systems as appropriate: Central Nervous System (CNS); eyes; ears, nose and throat (ENT); respiratory; cardiovascular; GI; musculoskeletal; neurological; endocrine/metabolic; Genitourinary; haematopoietic/lymphatic; dermatological.

The outcome of these assessments will be documented in the CRF and any abnormalities will be described. The Investigator can interpret individual findings based on the participant's age,

physical state and level of fitness. Participants with clinically significant findings outside the normal range should be excluded from the study based on investigator's discretion. This decision will be documented on the Comments page of the CRF.

6.6. Vital Signs

Qualified site staff will record participant's heart rate, **BLOOD PRESSURE** and oral body temperature when participants are resting in a sitting position, checked against the following normal ranges (which are given as a guide) and recorded in appropriate forms.

Heart rate:

60-100 beats per minute.

Oral body Temperature:

97.7–99.5 °F

Blood Pressure

Systolic: <140mmHg; Diastolic: <90mmHg

The Investigator can interpret individual findings based on the participant's age, physical state and level of fitness. Participants with clinically significant readings outside the normal range should be excluded from the study based on investigator's discretion. This decision will be documented on the Comments page of the eCRF.

6.7. Anthropometric Measurements (Height, Weight, and BMI)

All anthropometric measurements will be performed using the guidelines adopted at the National Institute of Health sponsored Arlie Conference (Lohman *et al.*, 1988).

Height will be measured using a portable stadio-meter (SECA 213), with the subject standing bare-foot, to the nearest 0.1 cm. An average of 3 measurements will be recorded.

Weight will be measured in standard clothing on standardised weighing scale (SECA ~~874~~ **803**) to the nearest 0.1 kg. Weight will be measured in the unit kg and an average of 3 measurements will be recorded.

6.8. Hb Assessment

A non invasive spectrophotometry based instrument (Masimo Pronto 7 Pulse CO-Oximeter) will be used for Hb assessment. The Masimo Pronto 7 Pulse CO-Oximeter uses signal extraction technology pulse oximetry.

Site staff will select an appropriate sized sensor. The sensor will be placed on the nail of ring finger of the left hand, followed by assessment of haemoglobin by pressing the test button. Appropriate care will be taken by the site staff to ensure that the participant's test finger nail does not contain any nail paint.

6.9. Laboratory assessment

Blood sample will be collected **FASTING 12 HOURS**, collection, transportation and storage will be done strictly according to lab manual **PROVIDED BY O2 LAB** for the respective test. **SITE WILL BE TRAINED BY LAB AT THE INITIATION VISIT REGARDING ALL OF THE ABOVE.**

AFTER APPLICATION OF AN ANESTHETIC PATCH/OINTMENT *E.G.* LIDOCAIN, TETRACAIN *ETC* TO REDUCE DISCOMFORT, A TOTAL VOLUME OF APPROXIMATELY 57.5mL (EACH AT BASELINE VISIT (2), MIDLINE (VISIT 4) AND ENDLINE (VISIT 6) TOTAL OF APPROXIMATELY 172.5mL) OF WHOLE BLOOD WILL BE COLLECTED FROM EACH PARTICIPANT BY A CERTIFIED PHLEBOTOMIST FOR BIOCHEMICAL AND MICRONUTRIENT ANALYSIS.

THE PARTICIPANT WILL BE MADE TO LIE DOWN ON BED OR SIT COMFORTABLY ON A CHAIR WITH HIS ARM STRETCHED ON THE TABLE. SHE WILL BE ASKED TO EXERCISE THE ARM SO AS TO MAKE THE ANTE-CUBITAL VEIN PROMINENT. A TOURNIQUET WILL BE TIED ABOUT 2 INCHES ABOVE THE VEIN ON THE UPPER ARM. THE VEIN WILL BE PALPATED, AND THE AREA WILL BE STERILISED. THE VEIN WILL BE PRICKED AND VACUTAINER PLACED IN POSITION TO COLLECT SAMPLE.

URINE SAMPLE COLLECTION.

SPOT URINE FOR TEST: CTX-1 URINE; URINARY CALCIUM AND CREATININE (CREATININE TO NORMALISE URINARY CALCIUM)

~~Collect second voided urine sample in the morning after an overnight fast. Site to aliquot 0.5ml of urine into transport tube and send frozen.~~

~~24 hours urine:~~

~~Patient is supplied with 24hr urine bottle and is provided by site staff information of when to start the collection. Patient must not use first void of the day to start collection. Patient collects urine in same bottle, and refrigerates after each void. The last sample collected must be the first specimen voided the following morning at the same time as previous days first void. Site to aliquot 10ml of urine into transport tube and send at ambient temperature.~~

~~The analytical method, assay validation, and analytical report for this study will be provided by the analytical investigator. In addition, the analytical report will include a statement regarding the stability of the frozen samples, limit of quantification, recovery, and a summary of the standard curves. In addition, the report will include representative chromatograms (if any).~~

IMMEDIATELY AFTER COLLECTION, SAMPLES WILL BE FROZEN AT OR BELOW -20°C (FOR SERUM CENTRIFUGE BEFORE STORAGE). IF FREEZING IS NOT POSSIBLE, THEY WILL BE REFRIGERATED IMMEDIATELY AT OR BELOW 4°C AND MAINTAINED AT THIS TEMPERATURE FOR NO LONGER THAN 2 HOURS BEFORE FREEZING ~~AT OR BELOW -70°C~~ BETWEEN -60^0 TO -80^0 C.

~~All samples (baseline and end line) will be analysed in batches.~~ **TESTS RUN AT THE BIOMARKER LAB WILL BE ANALYSED IN BATCHES FOR CTX-1 SERUM, cOC, uOC, CTX-1 URINE . OTHER SAMPLES WILL BE TESTED WHEN THEY ARE RECEIVED IN THE LAB.**

HBS TO BE STORED TILL IT IS PROPERLY DISPOSED OF AT THE END OF ITS RETENTION PERIOD (I.E. 14 DAYS FROM ~~LAST~~ EACH SAMPLE ANALYSIS ~~TILL STUDY REPORT IS ISSUED~~), OR UPON RECEIPT OF A REQUEST TO DESTROY THE HBS DUE TO WITHDRAWAL OF CONSENT.

~~Storage of biological sample will be held till 14 DAYS FROM LAST SAMPLE ANALYSED 2 month past final report is out,~~ longer storage is not advised because of stability of markers analyzed.

6.10. Dietary assessment (7day food diary)

The participants to register all food, water and beverage consumed during one complete week. The diary include (template in Appendix III) the different meal times (at waking up/before breakfast, during breakfast, during morning/before main meal, during main meal, during afternoon/before dinner, during dinner, and before going to bed), a space to specify the type of food item consumed, the amount consumed during the day **IN GRAMS** ~~both in mL and portion sizes using standard measure~~ **USING WEIGHING SCALE WENSAR KITCHEN BALANCE TTB 3 MODEL** given with diary, and the brand (in case it was a commercial food product). ~~To increase the accuracy of the reported portion size, interviewers will visit to report intake with the subjects at every visit using standard measurement cups.~~ Dietary assessment will be done using standard nutrition intake program **DIETSOFT SOFTWARE USING LATEST VERSION BASED NIN, INDIA FOOD GUIDE.**

6.11. Study Conclusion

Subjects will be evaluated to determine if they completed all study procedures or if they were discontinued from the study early. If the subject discontinued at any point during the study, the primary reason for withdrawal should be recorded on the Study Conclusion page of the CRF by selecting one of the options below.

1. Subject did not meet study criteria
2. Adverse Event
3. Lost to Follow Up
4. Protocol Violation
5. Withdrawal of Consent
6. Other

6.12. Clinical Safety Laboratory Assessments

All protocol required laboratory assessments, must be conducted in accordance with the Laboratory Manual or according to the laboratory section of the Protocol, and Protocol Schedule of Events. Samples must be clearly labeled with the subject number, protocol number, site/centre number, and visit date. Details for the preparation and shipment of samples will be provided by the laboratory. Reference ranges for all safety parameters will be provided to the site by the laboratory responsible for the assessments.

If additional non-protocol specified laboratory assessments are performed at the institution's local laboratory and result in a change in subject management or are considered clinically significant or abnormal by the investigator (e.g., SAE or AE or dose modification) the results must be recorded in the CRF. If the clinically significant abnormal lab is associated with a diagnosis, the diagnosis should be recorded on the CRF.

7. SAFETY ASSESSMENTS

7.1. Definitions of an Adverse Event and Serious Adverse Event

7.1.1. Adverse Events

The investigator or site staff will be responsible for detecting, documenting and reporting events that meet the definition of an AE or SAE.

Adverse Event Definition:
An AE is any untoward medical occurrence in a patient or clinical investigation subject, temporally associated with the use of an investigational or washout product, whether or not considered related to the investigational or washout product. NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of an investigational or washout product.

Events meeting AE definition include:
Any abnormal laboratory test results (if applicable) or other safety assessments, including those that worsen from baseline, and felt to be clinically significant in the medical and scientific judgment of the investigator. Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. New condition(s) detected or diagnosed after study product administration even though it may have been present prior to the start of the study. Signs, symptoms, or the clinical sequelae of a suspected interaction. Signs, symptoms, or the clinical sequelae of a suspected overdose of either study product or a concomitant medication (overdose per se will not be reported as an AE/SAE).

Events NOT meeting definition of an AE include:
Any clinically significant abnormal laboratory findings (if applicable) or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the subject's condition. The disease/disorder/ condition being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for

the subject's condition..

Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is an AE.

Situations where an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).

Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

7.1.2. Serious Adverse Events

Serious Adverse Event is defined as any untoward medical occurrence that, at any dose:
Results in death
Is life-threatening NOTE: The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject 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 hospitalization or prolongation of existing hospitalization NOTE: In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or out-patient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious. Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.
Results in disability/incapacity NOTE: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g. sprained ankle) which may interfere or prevent everyday life functions but do not constitute

a substantial disruption.

Is a congenital anomaly/birth defect

Other Situations

Medical or scientific judgment should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious.

Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization or development of drug dependency or drug abuse or reports of spontaneous abortion.

7.2. Recording Adverse Events and Serious Adverse Events

Recording of adverse events and serious adverse events:

The investigator or site staff will be responsible for detecting, documenting and reporting events that meet the definition of an AE or SAE.

The investigator or site staff will then record all relevant information regarding an AE/SAE in the CRF.

There may be instances when copies of medical records for certain cases are requested by GSK. In this instance, all subject identifiers, with the exception of the subject number, will be blinded on the copies of the medical records prior to submission of to GSK.

The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis will be documented as the AE/SAE and not the individual signs/symptoms. Clinical AEs will be described by diagnosis and not by symptoms when possible (e.g., upper respiratory tract infection, seasonal allergy, etc. instead of runny nose).

AEs will be collected from the start of the and until 7 days following last administration of the study product.

SAEs will be collected over the same time period as stated above for AEs. ~~**However, any SAEs assessed as related to study participation (e.g., investigational product, protocol mandated**~~

~~procedures, invasive tests, or change in existing therapy) or related to a GSK concomitant medication will be recorded from the time a subject consents to participate in the study up to and including any follow-up contact.~~

Medical conditions reported prior to the time period for reporting AEs/SAEs should be recorded as part of the subject's medical history.

7.3. Evaluating Adverse Events and Serious Adverse Events

Assessment of Intensity:

The investigator or designee will make an assessment of intensity for each AE and SAE reported during the study and will assign it to one of the following categories:

Mild: An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.

Moderate: An event that is sufficiently discomforting to interfere with normal everyday activities

Severe: An event that prevents normal everyday activities. - an AE that is assessed as severe will not be confused with an SAE. Severity is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.

Note: An event is defined as 'serious' when it meets at least one of the pre-defined outcomes as described in the definition of an SAE.

Assessment of Causality:

The investigator is obligated to assess the relationship between study product and the occurrence of each AE/SAE.

A "reasonable possibility" is meant to convey that there are facts/evidence or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.

The investigator will use clinical judgment to determine the relationship.

Alternative causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the study

product will be considered and investigated.

The investigator will also consult the Investigator Brochure (IB) and/or Product Information, for marketed products, in the determination of his/her assessment.

For each AE/SAE the investigator **must** document in the medical notes (source document) or CRF that he/she has reviewed the AE/SAE and has provided an assessment of causality.

There may be situations when an SAE has occurred and the investigator has minimal information to include in the initial report to GSK. **However, it is very important that the investigator always make an assessment of causality for every event prior to the initial transmission of the SAE data to GSK.**

The investigator may change his/her opinion of causality in light of follow-up information, amending the SAE data collection tool accordingly.

The causality assessment is one of the criteria used when determining regulatory reporting requirements.

7.4. Reporting Adverse Events and Serious Adverse Events

AE Reporting to GSKCH:

AEs will be recorded in the AE section of the CRF.

Medical conditions recorded by the subject on a diary card or similar document that meet the definition of an AE must also be recorded in the AE section of the CRF, if not previously well-characterized by the investigator in the subject's medical history.

AEs elicited by the investigator in a standard manner at the study visits should also be recorded in the AE section of the CRF. The investigator or designee must ask the subject the following question during each visit including any follow-up visits: ***"Have you felt unwell, experienced any symptoms or taken any medication (since your last visit) (today) (since your last dose) (since the last session)?"***

The medically qualified investigator should review adverse events in a timely manner; this review should be documented in writing in the source document or in the CRF.

After the study is completed at a given site, and the site has received their study data on Compact Discs (CDs), the electronic data collection tool will be removed from the internet to prevent the

entry of new data or changes to existing data.

SAE Reporting to GSKCH:

A paper copy of the SAE form provided in the investigator study master file should be completed as fully as possible.

It is essential to enter the following information:

Protocol and subject identifiers

Subject's demography

Description of events, with diagnosis if available

Investigator opinion of relationship to study product (see section 8.3)

Criterion for seriousness.

The following are desirable and are of particular relevance for investigator and GSKCH assessment of the SAE report:

Date of onset of AE

Date AE stopped, if relevant

Study product start date

Study product end date if relevant

Action taken on study product

Outcome if known

The SAE form, completed as fully as possible, and SAE fax cover sheet must be faxed or e-mailed to the appropriate GSKCH study manager as soon as possible, but not later than 24 hours after study site personnel learn of the event. The GSKCH study manager should be notified of the situation by telephone or email.

Fax serious adverse events to:

India: PPD

The GSKCH study manager will be responsible for forwarding the SAE form to the case management group, global clinical safety and phamacovigilance, the medical director responsible for the study and other GSKCH personnel as appropriate via email.

The initial report will be followed up with more information as relevant, or as requested by the GSKCH study manager.

7.5. Follow-up of Adverse Events and Serious Adverse Events

Follow-up of AEs and SAEs:

After the initial report, the investigator is required to proactively follow up with each subject and provide further information on the subject's condition.

All AEs/SAEs will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or until the subject is lost to follow-up.

The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as may be indicated or as requested by GSK to elucidate as fully as possible the nature and/or causality of the AE or SAE.

Investigators are not obliged to actively seek AEs or SAEs in former subjects. However, if the investigator learns of any SAE, including the death, at any time after a subject has been discharged from the study, and considers the event reasonably related to the investigational product or study participation, the investigator will promptly notify GSKCH.

The investigator will submit any updated SAE data to GSK within the designated reporting time frames.

Regulatory and ethics reporting requirements for SAEs:

The investigator will promptly report all SAEs to GSKCH within the designated reporting timeframes (within 24 hours of learning of the event). GSKCH has a legal responsibility to notify, as appropriate, the local regulatory authority and other regulatory authorities about the safety of a product under clinical investigation. Prompt notification of SAEs by the investigator to GSKCH is essential so that legal obligations and ethical responsibilities towards the safety of subjects are met.

GSKCH will comply with country specific regulatory requirements relating to safety

reporting to the regulatory authority, IRB/IEC and investigators.

Investigator safety reports are prepared according to GSKCH policy and are forwarded to investigators as necessary. An investigator safety report is prepared for a SAE(s) that is both attributable to investigational product and unexpected. The purpose of the report is to fulfill specific regulatory and GCP requirements, regarding the product under investigation.

An investigator who receives an investigator safety report describing a SAE(s) or other specific safety information (e.g., summary of listing of SAEs) from GSKCH will file it with the Investigator Brochure (or safety statement) and will notify the IEC, if appropriate according to local requirements.

7.7. Collection of Pregnancy Information

7.7.1. Time Period for Collecting of Pregnancy Information

Collection of Pregnancy Information:
Pregnancy information will be collected on all pregnancies reported following administration of any investigational product. Information on pregnancy identified during the screening phase and prior to investigational product administration does not need to be collected.

7.7.2. Action to be Taken if Pregnancy Occurs

Action to be Taken:
The investigator will collect pregnancy information on any subject who becomes pregnant while participating in the study after administration of the investigational product. The investigator will record pregnancy information on the appropriate form and submit it to GSKCH within 2 weeks of learning of the subject becoming pregnant. The subject will be followed to determine the outcome of the pregnancy. Information on the status of the mother and infant / neonate (including concomitant medications taken by the mother during the pregnancy) will be forwarded to GSKCH. Generally, follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported. While pregnancy itself is not considered to be an AE, any pregnancy complication or elective termination for medical reasons will be recorded as an AE or SAE.

A spontaneous abortion is always considered to be an SAE and will be reported as such. An SAE occurring in association with a pregnancy, brought to the investigator's attention after the subject completed the study and considered by the investigator as possibly related to the investigational product, must be promptly forwarded to GSK.

While the investigator is not obliged to actively seek this information in former study participants, he or she may learn of an SAE through spontaneous reporting.

If the subject becomes pregnant during the study they should be withdrawn from the study and this should be recorded in the appropriate section of the CRF.

8. DATA MANAGEMENT

For this study subject data will be entered into an electronic case report form, using a GSKCH validated data system.

8.1. Source Documents/ Data

The source documents (e.g. hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files and records kept at the pharmacy, at the laboratory and at the medico-technical departments involved in the clinical study) which contain the source of data recorded in the CRF should be specified in the Source Document Designation Form. In some cases the CRF can be used as a source document.

Each subject will be assigned and identified by a unique Screening Number. Any reference made to an individual subject within the study must be done using the unique Screening Number.

8.2. Electronic Case Report Form

A CRF is a printed, optical, or electronic document designed to record all of the protocol required information to be reported to the sponsor on each trial subject.

For each subject who has given informed consent/assent and has been screened, CRF must be completed and signed by the Principal Investigator (or authorized designee) to certify that the data are complete and correct.

Management of clinical data will be performed in accordance with applicable GSKCH standards and data cleaning procedures to ensure the integrity of the data e.g. removing errors and inconsistencies in the data.

In order to protect the privacy of subjects, no Personally Identifiable Information (PII) (including the subject's name or initials or birth date) is to be recorded in the CRF or as part of the query text.

Adverse events and concomitant medications terms (if applicable) will be coded using MedDRA (Medical Dictionary for Regulatory Activities) and an internal validated medication dictionary, GSKDrug.

Subject data will be entered into GSKCH defined CRFs and transmitted electronically to GSKCH in a validated (21 CFR Part 11 compliant) web-based electronic data capture system (InForm™).

All CRF pages should be completed during a subject assessment when the CRF has been designated as the source. Data that is sourced elsewhere should be entered into the CRF in an agreed upon timeframe between the Investigator and Sponsor.

The CRFs (including queries, query responses and audit trails) will be retained by GSKCH. Site data archived compact discs (CD(s)) prepared by a third party will be sent to the investigator to maintain as the investigator copy following the decommissioning of the study.

8.3. Data Handling

Documentation of all data management activities should allow step-by-step retrospective assessment of data quality and study performance. Any changes or corrections to data will be performed in the Electronic Data Capture (EDC) System, and it will include rationale for changes. The EDC system has an audit trail, which will provide a complete record of the changes and corrections endorsed by the Investigator.

8.3.1. Data Queries

Programmed edit checks will be generated automatically, as the data is being entered into the system. Data Management will also run reports and listings on the CRF data, in addition to the queries already programmed and generated by the system, to raise manual queries as needed for site clarification or correction. The Clinical Dictionary Development and Management Group will raise queries as needed on safety data to code the terms (Adverse Events and Drugs) are reported appropriately.

The study monitor at the study site will review the CRFs in accordance with the monitoring plan, and any queries will be generated in the EDC System to the Investigator or designee, enabling the errors to be addressed in parallel with Data Management review. Monitor can also run reports and listings on the CRFs, to raise manual queries as needed for site clarification or correction

8.4. Processing Patient Reported Outcomes

Patient reported outcome (PRO) data are collected directly from the subject PRO measures e.g. diary cards, questionnaires etc, and entered into the sponsor's clinical data management system (DMS) by the study site representative. In instances where the PRO data is entered into the DMS by GSKCH, the PROs will be anonymised, and forwarded to GSKCH for entry, as agreed and documented ahead of the study starting. PROs that are source will be retained by the investigator and certified copies will be sent to GSKCH.

In order to protect the privacy of participants, no Personally Identifiable Information (PII) (including the subject's name or initials or birth date) is to be recorded on all PRO's that will be forwarded to GSKCH.

8.5. External Data

External Data are subject data obtained externally to the CRF. These data are generated from laboratory instruments, computers or other sources and then transcribed into a file and format agreed upon by GSKCH to identify the subject and time point referenced in the CRF and/or protocol.

An agreed upon quality control process is performed against the transcribed data to the source to ensure the accuracy of the transcription. The transcribed data is transmitted in an agreed upon format to GSKCH via secured web portal or CD/DVD via mail carrier with tracking capabilities.

Proper reconciliation will be performed between the transcribed data and the clinical database to ensure subject and time point referenced in the Clinical Database match before Clinical Database Freeze (locking of the database) can occur.

9. STATISTICAL CONSIDERATIONS AND DATA ANALYSES

9.1 Sample Size Determination

The first primary objective for this study is changes in s-CTX-1 levels. Naylor et al. (2015) used bone turnover markers to identify women who responded to bisphosphonate treatment for osteoporosis. The change in CTX with risedronate (above the effect of the calcium and vitamin D supplement) was 30%. Thus, we can say that a 30% reduction has been shown to be associated with a reduction in fractures. Hence, a reduction of 20% in test product as compared to placebo could be considered as a clinically significant difference.

Estimates of mean s-CTX-1 and variability from Kruger et al, 2006 were as follows:

Estimates of s-CTX-1 and variability from Kruger et al, 2006 (Mean ± SD[1])

Endpoint		Test product	Control Product
CTX-1	Baseline	0.45 ± 0.166	0.44 ± 0.032
	Post-Baseline (Week16)	0.30 ± 0.094	0.46 ± 0.092
	Change from baseline	-0.15 ± 0.144	0.02 ± 0.142

1: SE values were reported in the paper. SD was calculated as SE*SQRT (N).

SD values for change from baseline were not reported in the paper. These were calculated using following formula considering correlation coefficient between baseline and post-baseline values as 0.5:

$$SD_{E,change} = \sqrt{SD^2_{E,baseline} + SD^2_{E,final} - (2 \times Corr \times SD_{E,baseline} \times SD_{E,final})}$$

$SD_{E,Change}$ = SD for change from baseline

$SD_{E,Baseline}$ = SD for baseline

$SD_{E,final}$ = SD for post-baseline

Corr = correlation coefficient between baseline and final values

Considering similar baseline values and 20% clinically relevant decrease in test product (lower values are better) from baseline, -0.09 will be used as change from baseline value in test product. This gives a treatment difference of -0.11.

Based on the above estimates of treatment difference and taking the larger estimate for variability (i.e SD=0.144), to be able to achieve 90% power, 44 participants per treatment arm (Total =88) will be required to complete the study. This assumes 0.025% level of significance (two tailed t-test).

To allow for 20% drop-out rate, a total of 54 participants (Total=108) will be randomised per treatment arm.

The co-primary objective for this study is changes in (c-OC/uc-OC) levels. From Binkley et al. the following data related to the end point was received.

From the estimates of %uc-OC, and Total OC from Binkley et al (Mean), the estimates of c-OC have been calculated. From the available data the following table has been tabulated.

Endpoint		Test product	Control Product
c-OC/uc-OC	Baseline	11.7206	11.96
	Post-Baseline (Week2)	31.868	18.60
	Change from baseline	20.14	7.53

Considering the delta value as 12.5 and the SD as 13 using Taylor series expansion the sample size to be able to achieve 90% power, 30 participants per treatment arm (Total =60) will be required to complete the study. This assumes 0.025% level of significance (two tailed t-test).

To allow for 20% drop-out rate, a total of 36 participants (Total=72) will be randomised per treatment arm.

However, the sample size for the first primary end point is larger so the sample size will be required for this study is 108 in total.

The sample size is based on the primary endpoint. No sample size considerations have been taken into account for secondary objectives.

Approximately 135 participants will be screened to randomise approximately 108 participants in order to obtain 88 participants completing the study.

Following assumptions have been taken for sample size estimation:

Estimates of s-CTX-1 will be similar in Indian Women as compared to New Zealand Population (Kruger et al, 2006).

Product used in Kruger et al, 2006 and in the proposed study will provide similar estimates of s-CTX-1.

Values at 6 months in the proposed study will be similar to values at 4 months from Kruger et al, 2006.

Women 25-35 years old will have similar estimates of s-CTX-1 as 36-45 years old from Kruger et al.

Vitamin K supplementation reduces serum concentrations of under carboxylated osteocalcin in healthy young and elderly adults from Binkley et al.

9.2. General Considerations

9.2.1. Definition of Analysis Populations

All analyses of safety will be made on the safety population which will be defined as all subjects who are randomized and have received at least one dose of study treatment. The safety population will be analyzed as per treatment received.

The intent-to-treat (ITT) population which will be defined as all randomized subjects with at least one post treatment efficacy assessment.

The pre-protocol (PP) population is defined as those subjects in the ITT population for whom all post baseline efficacy measures are not deemed to be affected by protocol violations.

Statistical analyses of the efficacy parameters will be based on the ITT population. The PP analysis will be performed for the primary endpoint if more than 10% of the subjects in the ITT population are removed from the PP population. Post baseline efficacy assessments deemed to be affected by protocol violations will be removed for the per-protocol analysis.

9.2.2. Exclusion of Data from Analysis

The following will be reviewed and those protocol violations considered to affect efficacy assessments will lead to exclusion of either subject or data from PP analyses:

Violation of inclusion or exclusion criteria

Significant non compliance with assigned treatment

Efficacy assessments outside the specified time windows

Use of prohibited treatment or medication before or during the study

Any other reason identified which may affect the efficacy assessments.

All protocol violations and their impact on efficacy analyses will be determined between the Biostatistician and Clinical Research Director or designee, ahead of database lock and unblinding.

9.2.4. Criteria for Assessing Efficacy

The primary success criteria is to observe a statistically significantly greater reduction in s-CTX-1 for the test product than the reference product or to observe a statistically significantly greater increase in c-OC/uc-OC for the test product than the reference product, after 6 months of treatment.

9.2.5. Criteria for Assessing Tolerability

The assessment for safety will be based on the AEs reported by subjects following dosing with study treatment.

9.3. Statistical Methods and Analytical Plan

Additional details of the proposed statistical analysis will be documented in the statistical analysis plan (SAP), which will be written following finalization of the protocol and prior to study unblinding.

9.3.1. Demographic and Baseline Characteristics

Descriptive statistics (number of subjects, mean, standard deviation, median, minimum and maximum for continuous variables, and frequency and percentage for categorical variables) will be provided for demographic and baseline data.

9.3.2. Primary Analysis

The co-primary efficacy variables of the study are the change from baseline in bone resorption marker (s-CTX-1) after 6 months and the change from baseline in the ratio of carboxylated to under carboxylated osteaocalcin (c-oC/uc-OC) after 6 months.

Each of these co-primary efficacy variables will be analyzed using the analysis of covariance (ANCOVA). The ANCOVA will have treatment as fixed effect and the corresponding baseline as covariate. Adjusted means, 95% confidence intervals, within treatment p-values for each treatment group, treatment group difference, 95% confidence interval of the difference and the between-treatment p-values based on the statistical model described above will also be presented. Statistical tests to compare treatments will be two-sided and will employ a level of significance of $\alpha = 0.025$.

The setting of the significance level of 0.025 for these 2 co-primary endpoints will ensure that the overall significance level for the primary endpoint is less than or equal to 0.05.

Assumptions of normality and homogeneity of variances will be evaluated. If violations are observed then suitable data transformations will be tried to achieve the assumptions. If suitable transformations cannot be found, non-parametric analyses will be performed.

9.3.3. Secondary Analysis

The secondary efficacy variables are:

Change from baseline in s-CTX-1 after 3 months of treatment

Change from baseline in c-OC/uc-OC after 3 months of treatment

Change from baseline in urinary CTX-1 after 3 and 6 months of treatment

Change from baseline in s-NTX-1 after 3 and 6 months of treatment

Change from baseline in s-P1NP after 3 and 6 months of treatment

Change from baseline in BSAP after 3 and 6 months of treatment

Change from baseline in s-PTH after 3 and 6 months of treatment

Change from baseline in Urinary Calcium **USING CREATININE IN SPOT TEST TO NORMALISE** after 3 and 6 months of treatment

Change from baseline in Serum Ca after 3 and 6 months of treatment

Change from baseline in Serum P after 3 and 6 months of treatment

Change from baseline in Serum ALP after 3 and 6 months of treatment

Change from baseline in 25 OH D3 after 3 and 6 months of treatment

Change from baseline in Serum Se after 3 and 6 months of treatment

Change from baseline in ~~Serum~~ **PLASMA** Zn after 3 and 6 months of treatment

Change from baseline in Serum Folic acid after 3 and 6 months of treatment

Change from baseline in ~~Serum~~ **PLASMA** B6 after 3 and 6 months of treatment

Change from baseline in Serum B12 after 3 and 6 months oftreatment

Secondary efficacy variables will be analysed in a similar way as the co-primary efficacy variables. The only difference will be that all significance levels for testing of hypotheses will be 5%.

9.3.3. Exploratory Analysis

The change from baseline in IGF-1 after 3 and 6 months of treatment will be analysed in a similar way as the co-primary efficacy variables, the only difference being that the significance level will be 5% for all tests of hypotheses.

9.3.4. Safety Analysis

Treatment emergent AEs and treatment emergent treatment related AEs will be summarised. AEs will be considered treatment emergent if they occur on or after the date and time of the first dose of study treatment.

AEs will be listed. No inferential analyses will be performed to compare treatments with respect to safety data.

10. STUDY GOVERNANCE CONSIDERATIONS

10.1. Posting of Information on Publicly Available Clinical Trials Registers

Study information from this protocol will be posted on publicly available clinical trial registers before enrollment of subjects begins.

10.2. Regulatory and Ethical Considerations, Including the Informed Consent

The study will be conducted in accordance with all applicable regulatory requirements, and with GSK policy.

The study will also be conducted in accordance with ICH Good Clinical Practice (GCP), all applicable subject privacy requirements, and the guiding principles of the current version of the Declaration of Helsinki. This includes, but is not limited to, the following:

Before initiating a trial, the investigator/institution should have written and dated approval/favorable opinion from the IEC for the trial protocol (including amendments), written informed consent form, consent form updates, subject recruitment procedures (e.g., advertisements), investigator brochure/ safety statement (including any updates) and any other written information to be provided to subjects. A letter or certificate of approval will be sent by the investigator to the sponsor prior to initiation of the study, and also when subsequent amendments to the protocol are made.

Signed informed consent to be obtained for each subject before participation in the study (and for amendments as applicable)

Investigator reporting requirements (e.g. reporting of AEs/SAEs/protocol deviations to IEC

GSK will provide full details of the above procedures, either verbally, in writing, or both.

10.3. Quality Control (Study Monitoring)

In accordance with applicable regulations including GCP, and GSK procedures, GSK or designee (i.e. third party vendor) monitors will contact the site prior to the start of the study to

review with the site staff the protocol, study requirements, and their responsibilities to satisfy regulatory, ethical, and GSK requirements.

When reviewing data collection procedures, the discussion will include identification, agreement and documentation of data items for which the CRF will serve as the source document.

GSK or designee will monitor the study and site activity to verify that the:

Data are authentic, accurate, and complete.

Safety and rights of subjects are being protected.

Study is conducted in accordance with the currently approved protocol and any other study agreements, GCP, and all applicable regulatory requirements.

The extent and nature of monitoring will be described in a written monitoring plan on file at GSKCH. The investigator (or designee) agrees to allow the monitor direct access to all relevant documents and agrees to co-operate with the monitor to ensure that any problems detected in the course of these monitoring visits are resolved.

10.4. Quality Assurance

To ensure compliance with GCP and all applicable regulatory requirements, GSK may conduct a quality assurance assessment and/or audit of the site records, and the regulatory agencies may conduct a regulatory inspection at any time during or after completion of the study.

In the event of an assessment, audit or inspection, the investigator (and institution) must agree to grant the advisor(s), auditor(s) and inspector(s) direct access to all relevant documents and to allocate their time and the time of their staff to discuss the conduct of the study, any findings/relevant issues and to implement any corrective and/or preventative actions to address any findings/issues identified.

The sponsor will be available to help investigators prepare for an inspection.

10.5. Conditions for Terminating the Study

Upon completion or premature discontinuation of the study, the GSKCH monitor will conduct site closure activities with the investigator or site staff, as appropriate, in accordance with applicable regulations including GCP, and GSKCH Standard Operating Procedures.

Both GSKCH and the Investigator reserve the right to temporarily suspend or prematurely discontinue this study at any time for reasons including, but not limited to, safety or ethical issues or severe non-compliance. For multicenter studies (if applicable), this can occur at one or more or at all sites.

If the trial is prematurely terminated or suspended for any reason, the investigator site should promptly inform the trial subjects and should assure appropriate therapy/ follow-up for the subjects. Where required by the applicable regulatory requirements, GSKCH should inform the regulatory authority(ies).

In addition:

If the investigator terminates or suspends a trial without prior agreement of GSKCH, the investigator site should promptly inform the sponsor and the IEC, and should provide the sponsor and the IEC a detailed written explanation of the termination or suspension.

If the GSKCH terminates or suspends a trial, the investigator should promptly inform the IEC and provide the IEC a detailed written explanation of the termination or suspension.

If the IEC terminates or suspends its approval/favorable opinion of a trial, the investigator should promptly notify the GSKCH and provide GSKCH with a detailed written explanation of the termination or suspension.

10.6. Records Retention

Following closure of the study, the investigator must maintain all site study records (except for those required by local regulations to be maintained elsewhere), in a safe and secure location.

The records (study/ site master file) must be maintained to allow easy and timely retrieval, when needed (e.g., for a GSK audit or regulatory inspection) and must be available for review in conjunction with assessment of the facility, supporting systems, and relevant site staff.

Where permitted by local laws/regulations or institutional policy, some or all of these records can be maintained in a format other than hard copy (e.g., microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken.

The investigator must ensure that all reproductions are legible and are a true and accurate copy of the original and meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the investigator must ensure there is an acceptable back-up of these reproductions and that an acceptable quality control process exists for making these reproductions.

The investigator must assure that the subject's anonymity will be maintained. On CRFs or other documents submitted to GSKCH, subjects should not be identified by their names or initials, but by an identification code. The investigator should keep a separate log of subjects' codes, names and addresses. Documents not for submission to GSKCH, e.g. subjects' written consent forms, should be maintained by the investigator in strict confidence.

GSK will inform the investigator of the time period for retaining these records to comply with all applicable regulatory requirements (GSKCH recommends that documents be kept for 10 years). The investigator is also required to keep subject identification codes on file for at least 15 years after completion or discontinuation of the study. The minimum retention time will meet the strictest standard applicable to that site for the study, as dictated by any institutional requirements or local laws or regulations, GSK standards/procedures, and/or institutional requirements.

No study document should be destroyed without a prior written agreement between GSKCH and the investigator. The investigator must notify GSK of any changes in the archival arrangements, including, but not limited to, archival at an off-site facility or transfer of ownership of the records in the event the investigator is no longer associated with the site.

10.7. Provision of Study Results to Investigators, posting of Information on Publicly Available Clinical Trials Registers and Publication

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the clinical study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results at a GSK site or other mutually-agreeable location.

GSK will also provide the investigator with the full summary of the study results. The investigator is encouraged to share the summary results with the study subjects, as appropriate.

The procedures and timing for public disclosure of the results summary and for development of a manuscript for publication will be in accordance with GSK Policy.

A manuscript will be progressed for publication in the scientific literature if the results provide important scientific or medical knowledge.

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12. APPENDICES

12.1. Appendix 1 – Product Nutritional Profile

Nutritional Composition of Test and Placebo		Test- Nutritional Supplement		Placebo
Nutrient	ICMR/WHO RDA	Amount (per daily serve, 60g)	% RDA	**AMOUNT (PER DAILY SERVE, 60G)**
Energy	NA	215.9 Kcal	NA	215.9 Kcal
Carbohydrate Of which sugar	NA	40.9 gm 0	NA	47gm 10.8 gm (approx)
Fat Total Protein (good quality protein)	NA	2 gm (max) 9g ~~(6g)~~	NA	2 gm(max) **3 gm (max)** ~~2-5 g% (TBD)~~
Vitamin A	600 mcg	198	33	
Vitamin D	400 IU (10 mcg)	400 IU (10 mcg)	100	
Vitamin E	7.5 mcg	2.5	33	
Vitamin K2-7	55 mcg	55	100	
Vitamin B1	1 mg	0.33	33	
Vitamins B2	1.1 mg	1.1	100	

Nutritional Composition of Test and Placebo		Test- Nutritional Supplement		Placebo
Niacin	12 mg	4	33	
Vitamin B6	2 mg	2	100	
Vitamin B12	1 mcg	1	100	
Folic acid	120 mcg	120 mcg	100	
Biotin	30 mcg	9.9	33	
Pantothenic acid	5 mg	1.7	33.6	
Vitamin C	40 mg	40	100	
Calcium	600 mg	600	100	
Iodine	150 mcg	49.5	33	
Magnesium	310 mg	72.6	23	
Zinc	10 mg	1.62	16.2	
Selenium	26 mcg	26	100	
POTASSIUM	**3225**	**325.01**	**10.08**	

12.2. Appendix II - Abbreviations

AE	Adverse Event

CD	Compact Disc
CRF	Case Report Form
EDC	Electronic Data Capture
GCP	Good Clinical Practice
GSKCH	GlaxoSmithKline Consumer Healthcare
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ITT	Intention to Treat
PII	Personally Identifiable Information
PP	Per Protocol
SAE	Serious Adverse Event
PRO	Patient Reported Outcome
s-CTX-1	Serum C-terminal cross-linking telopeptide of type I collagen
NTX-1	N-terminal cross-linking telopeptide of type I collagen
CTX-1	C-terminal cross-linking telopeptide of type I collagen
s-P1NP	N-terminal propeptide of type I procollagen
s-OC	Serum Osteocalcin
s-uc OC	Serum under-carboxylated osteocalcin
s-PTH	Serum Parathyroid Hormone

12.3. Appendix III- 7 day food diary template

Week#	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Notes	Comments
8:00:00									
9:00:00									
10:00:00									
11:00:00									
12:00:00									
13:00:00									
14:00:00									
15:00:00									
16:00:00									

17:00:00									
18:00:00									
19:00:00									
20:00:00									
21:00:00									
22:00:00									
23:00:00									
24:00:00									

Instruction to use diary: The participants to register all food, water and beverage consumed during one complete week. The diary include the different meal times (at waking up/before breakfast, during breakfast, during morning/before main meal, during main meal, during afternoon/before dinner, during dinner, and before going to bed), a space to specify the type of food item consumed, the amount consumed during the day (both in mL and portion sizes using standard measure given with diary), and the brand (in case it was a commercial food product). To increase the accuracy of the reported portion size, interviewers will visit to report intake with the subjects at every visit using standard measurement cups, **BOWL AND SPOON**.

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SIGNATURE PAGE

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