

### Biochemical measurements from cohort samples of Fracture Study

35 assays of 400 randomly selected cohort samples were performed between June 1994 to June 1995 by 5 laboratories:

1. Endocrine Science, Calabasas Hill, CA
2. INSERM Research Unit 403 "Pathophysiology of Osteoporosis", Edouard Herriot Hospital, Lyon, France
3. Center for Clinical and Basic Research, Denmark
4. Laboratory for Clinical Biochemistry Research, University of Vermont, Colchester, VT
5. Calcitropic Hormone Reference Laboratory, University of California, San Francisco, San Francisco, CA

The assays measured in each laboratory are as follows:

Laboratory	Assay
Endocrine Sciences	Estradiol (total) <sup>1</sup>
	Estrone (total) <sup>1</sup>
	Free Testosterone <sup>1</sup>
	IGF-1
	IGF-binding protein-3
	% Free Testosterone <sup>1</sup>
	Sex Hormone Binding Globulin <sup>1</sup>
	Total Testosterone <sup>1</sup>
	TSH <sup>2</sup>
	Cholesterol <sup>2</sup>
	Triglycerides <sup>2</sup>
	HDL <sup>2</sup>
	DHEA-S <sup>3</sup>
	Fructosamine <sup>4</sup>
	Vitamin B-12 <sup>5</sup>
	3 alpha-diol G
Ferritin	
T4	
Free T4	
UCSF Calcitropic Hormone Lab	1,25(OH)2D <sup>1</sup>
	25(OH)D <sup>1</sup>
	Calcium <sup>1</sup>
	Creatinine <sup>6</sup>
	PTH <sup>1</sup>
Edouard Herriot Hospital, INSERM Research	Bone-specific Alkaline Phosphatase <sup>7</sup>
	Osteocalcin <sup>7</sup>
Center for Clinical and Basic Research	CTX <sup>7</sup>

<b>Laboratory</b>	<b>Assay</b>
University of Vermont Laboratory for Clinical Biochemistry Research	Albumin <sup>4</sup>
	Alpha1
	Alpha2
	Beta
	C-reactive protein
	Gamma
	Total protein <sup>4</sup>

Insulin-like growth factor -1 (IGF-1) and insulin-like growth factor binding protein-3 (IGFBP-3) were measured in serum by radioimmunoassay. For IGF-1, the intraassay coefficient of variation ranged from 20% at 26 ng/mL to 4.6% at 603 ng/mL; the interassay coefficient of variation ranged from 28% at 24 ng/mL to 6.3% at 580 ng/mL. For IGFBP-3, the intraassay coefficient of variation ranged from 5.1% to 13%; the interassay coefficient of variation ranged from 5.5% to 18%.

3 alpha-androstenediol glucuronide (3 alpha-diol G) in serum was measured by radioimmunoassay. The interassay coefficient of variation ranged from 9.9% to 40%.

Ferritin was measured in serum based on the Microparticle Enzyme ImmunoAssay (MEIA) technology. The intraassay coefficient of variation ranged from 1.8% to 4.9%. The interassay coefficient of variation ranged from 3.3% to 6.2%.

Free thyroxine (free T4) was measured in serum by radioimmunoassay. The intraassay coefficient of variation ranged from 10.7% to 12.3%. The interassay coefficient of variation ranged from 15.1% to 15.3%.

C-reactive protein (CRP) was measured by a colorimetric competitive ELISA developed in University of Vermont (Macy, et al, 1997). Biotinylated CRP competes with CRP in the sample for the coated antibody. Detection is via the enzyme horseradish peroxidase conjugated in an avidin-biotin complex followed by the color reagent substrate, orthophenylene diamine. The analytical CV for this assay is 5.14%. The expected normal range is 0.18 to 5.05 ug/mL.

Total protein, Albumin, Alpha1, Alpha2, Beta, and Gamma were measured by serum protein electrophoresis using a commercially available kit (Beckman Instruments, Brea, California). The reference range and interassay coefficient of variation for each as follows:

	<b>Reference range</b>	<b>CV</b>
Total protein	6.0 – 8.5 gm/dL	
Albumin	49 - 61%	5.3%
Alpha 1	2.4 - 4.9%	7.8%
Alpha 2	10 - 19%	6.6%
Beta	9 – 14%	6.3%
Gamma	11 – 21%	8.2%

Missing values may be due to 1) insufficient volume, 2) lower than detection value, 3) measurements not performed or 4) other reasons.

For more information on method, CV and sensitivity on assays which had been published, please referred to:

Reference:

1. Stone K, Bauer DC, Black DM, Sklarin P, Ensrud KE, Cummings SR. Hormonal predictors of bone loss in elderly women: a prospective study. The Study of Osteoporotic Fractures Research Group. *J Bone Miner Res.* 1998 Jul;13(7):1167-74.
2. Bauer DC, Ettinger B, Browner WS. Thyroid functions and serum lipids in older women: a population-based study. *Am J Med.* 1998 Jun;104(6):546-51.
3. Yaffe K, Ettinger B, Pressman A, Seeley D, Whooley M, Schaefer C, Cummings S. Neuropsychiatric function and dehydroepiandrosterone sulfate in elderly women: a prospective study. *Biol Psychiatry.* 1998 May 1;43(9):694-700.
4. Jamal SA, Stone K, Browner WS, Ensrud KE, Cummings SR. Serum fructosamine level and the risk of hip fracture in elderly women: a case-cohort study within the study of osteoporotic fractures. *Am J Med.* 1998 Dec;105(6):488-93.
5. Stone KL, Bauer DC, Sellmeyer D, Cummings SR. Low serum vitamin B-12 levels are associated with increased hip bone loss in older women: a prospective study. *J Clin Endocrinol Metab.* 2004 Mar;89(3):1217-21.
6. Ensrud K, Lui LY, Taylor BC, Ishani A, Shlipak MG, Stone KL, Cauley JA, Jamal SA, Antonucci DM, Cummings SR; Osteoporotic Fracture Research Group. Renal function and risk of hip and vertebral fractures in older women. *Arch Intern Med.* 2007 Jan 22;167(2):133-9.
7. Chapurlat RD, Bauer DC, Cummings SR. Association between endogenous hormones and sex hormone-binding globulin and bone turnover in older women: study of osteoporotic fractures. *Bone.* 2001 Oct;29(4):381-7.
8. Macy E, Hayes T, Tracy R (1997) Variability in the measurement of C-reactive Protein in Healthy subjects: implication for reference interval and epidemiological applications. *Clin Chem* 43:52-58.