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Therapeutic Implications of Vitamin D and Calcium in Overweight Women with Polycystic Ovary Syndrome

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Abstract

Objective—To assess effects of vitamin D and Calcium (Ca) on hormonal and metabolic milieu of polycystic ovary syndrome (PCOS)

Design—Single arm open label trial

Methods—Twelve overweight and vitamin D deficient women with PCOS underwent a 2 hour oral glucose tolerance testing at baseline and following 3 month supplementation with vitamin D (daily dose of 3533 IU, increased to 8533 IU after the first 5 participants) and 530mg elemental Ca daily.

Main Outcome Measures—Blood pressure (BP), plasma glucose, insulin, total testosterone (T) androstenedione (A), sex hormone binding globulin, lifestyle parameters were assessed at baseline and following 3 months intervention. Insulin resistance and AUC for glucose and insulin were computed; paired analyses were conducted.

Results—Improved serum 25OHD ($p<0.001$) and reductions in total T ($p=0.036$) and A ($p=0.090$) levels were noted following 3 month supplementation, compared to baseline. Significant lowering in BP parameters was seen in participants with baseline BP ≥ 120/80 mmHg (n=8) and in those with baseline serum 25OHD ≤ 20ng/ml (n=9). Parameters of glucose homeostasis and insulin resistance remained unchanged ($p>0.05$).

Conclusions—Androgen and BP profiles improved followed three month intervention, suggesting therapeutic implications of vitamin D and Ca in overweight and vitamin D deficient women with PCOS.

Keywords

Polycystic ovary syndrome; testosterone; androgen; vitamin D; calcium; insulin resistance

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Introduction

Polycystic Ovarian Syndrome (PCOS) is a recognized risk for cardiovascular disease and diabetes [1–4]. Limited data suggest pathophysiological relevance of vitamin D deficiency for PCOS [5–13]. Data from a pilot study of vitamin D and calcium (Ca) supplementation in a community sample of overweight and vitamin D deficient women with PCOS are presented.

Methods

The study was posted on clinicaltrials.gov (NCT00743574) [14]; institutional approval and written consent were obtained. Oligomenorrhea (menstrual cycles >35 days) and hyperandrogenism defined PCOS [15]. Serum 25OHD <25ng/ml and body mass index (BMI) ≥ 27 kg/m^2 were eligibility criteria; thyroid dysfunction, hyperprolactinemia, late onset congenital adrenal hyperplasia, use of insulin sensitizers, oral contraceptives, anti-epileptics, vitamin D and Ca supplements and known systemic disorders (e.g. hypertension, diabetes, urolithiasis, inflammatory bowel disease) were exclusion criteria.

Blood draw and anthropometric measurements were undertaken at baseline and after 3 month supplementation with study drugs. Serum 25OHD was tested after 1 month to ensure against toxicity (level>150ng/ml). Oral glucose tolerance testing (OGTT) was scheduled within 5 days of onset of bleeding (spontaneous or provoked following a 10 day course of oral progesterone) after overnight fast. BMI, waist circumference (WC, cm) and blood pressure (BP) were recorded. Serial blood samples were collected (fasting, 30, 60, 90 and 120 minutes after 75 gram oral glucose load [Trutol, Thermo Fisher Scientific Inc. RI]). Plasma glucose (G) levels were analyzed by glucose oxidase method and sample aliquots were stored at −80°C for assessment of insulin (I, Millipore RIA, St. Charles MO, sensitivity: 2uU/ml), total testosterone (T, IRA DSL Webster TX. sensitivity: 5 ng/dl), Androstenedione (A, ELISA, ALPCO, Salem, NH, sensitivity: 4ng/dl) and sex hormone binding globulin (SHBG, EIA, ALPCO, sensitivity: 0.1nmol/L). Free androgen index (FAI) was calculated. 25OHD was assessed by RIA (Diasorin Stillwater, MN, sensitivity 5ng/ml).

The study aimed to provide daily vitamin D3 (2000IU) and *monthly* vitamin D2 (50,000IU); D2 regimen was modified to 50,000IU *weekly* after observation of a suboptimal rise in serum 25OHD in the first 5 participants. All received 530mg of elemental Ca/day. Compliance was verified by pill count and tolerance was assessed.

Physical activity was determined by BDDS Adult PA Screener (www.nutritionquest.com) [16]; analyses provided estimates of total calories “expended” per day, and “MET minutes” per day at moderate and vigorous activity levels.

Statistics

Correlation analyses (Pearson’s or Spearman) assessed relationships between continuous data. Systolic BP (SBP) ≥ 140mmHg and/or diastolic BP (DBP) ≥ 90mmHg defined *hypertension*. Fasting G:I ratio, HOMA (Homeostatic model assessment), QUICKI (Quantitative insulin sensitivity check index), and area under the curve (AUC) for G and I were computed to assess insulin resistance (IR) [17]. Paired analyses compared baseline and completion data utilizing STATA 10 (College Station, TX); two tailed p ≤0.05 reflects statistically significance.

Results

Figure 1 provides enrollment overview (8-2008 to 3-2010); 80% of those initiating study drugs completed the trial (12/15). Compliance was 100% for vitamin D2, and between 80 and 90% for D3 and Ca. No adverse effects were noted.

Participant characteristics and data from baseline and following 3- month intervention are presented in Table I (cohort data) and II (individual participant data).

Serum 25OHD levels following 1 and 3 months of supplementation were significantly higher compared to baseline (26.17±4.82 and 28.58±6.30 ng/ml respectively compared to 17.58 ±4.56ng/ml at baseline, $p<0.001$); the observed changes in 25OHD were comparable between vitamin D dosing regimens (data not shown).

Lowering in SBP, DBP, and mean arterial BP (MAP) was observed compared to baseline (Table 1). Significant improvements in BP parameters were observed in those with SBP ≥120mmHg and/or DBP ≥80mmHg at baseline (n= 8, SBP 129.37 ± 5.75 versus 116.5 ±8.57 mmHg, p= 0.010; DBP 81.75 ± 7.57 versus 71.87 ±5.94 mmHg, p= 0.014); significant lowering in SBP was seen in those with baseline serum 25OHD ≤20ng/ml (n= 9, 122.22 ± 11.19 versus 112.56 ±10.42 mmHg, p= 0.033). At baseline, 17% (2/12) of the previously undiagnosed met criteria for *hypertension* compared to 0% at trial completion (p< 0.001, Fisher's exact test).

Significant reduction in total T and lowering in serum A were observed compared to baseline values (Table I). Indices of G homeostasis, BMI and WC were unaltered (Table I), as was physical activity (p> 0.05, data not shown).

Discussion

Vitamin D insufficiency is a modifiable risk factor for atherogenesis and hypertensive disorders [18–19] and our pilot data support this impression. Given that as little as 2mmHg decrease in SBP has been suggested to reduce CVD related morbidity and morbidity by almost 6% and all cause mortality by 3% [20], the observed mean reduction in SBP by 7mm is clinically meaningful. A lowering in SBP was previously described with 800IU of vitamin D and Ca in a cohort of elderly women [21], and we observed similar effects, albeit in a younger population.

The observed decline in androgens following 3 month supplementation with vitamin D and Ca is of interest. Reduction in total T and A (by 12 and 17% respectively) is comparable in magnitude to effect described with metformin use [22]. Since IR parameters were unaltered in our population, direct effects of vitamin D and Ca supplementation on the steroidogenesis pathway (ovarian and/or adrenal) can be hypothesized to explain the observed reduction in circulating androgens.

Although literature supports facilitatory influences of vitamin D on IR [23–25], our data fail to demonstrate any such effect. Selimoglu et al. [13], in contrast showed improvement in HOMA indices within 3 weeks of a single oral *mega* dose of 300,000 IU D3 in a pilot study of 11 women with PCOS, but did not observe any effect on androgens. A differential in the prevalence of morbid obesity, in the employed doses, and variations in the drug formulations may explain these contrasting findings; power constraint remains a plausible explanation. While the indices of IR were unaltered in our population, improvements in G homeostasis are suggested (participant #3, 4, 5 and 11) implying that perhaps the benefit of vitamin D supplementation may lie in mitigating progression of overt diabetes or in abnormalities of G

homeostasis; a need for cautious interpretation however is underscored by evidence of deterioration in G homeostasis in participant#2 (Table II).

The small sample size, non-randomized design and variable dosing are limitations of this study; stratified analyses however failed to identify a dose related differential in studied outcomes (data not shown). Our study design precludes from assessing effects of supplementation on hirsutism and acne; longer trial duration may have allowed study of effects of intervention on signs of hyperandrogenism.

In conclusion, our pilot data suggest potential therapeutic benefits of vitamin D and Ca supplementation in ameliorating the hormonal milieu and PCOS related sequelae in women deficient in vitamin D. Appropriately designed future studies are needed to better explore the proposed benefits of vitamin D and Ca for clinical stigmata of PCOS. Given that millions are affected by PCOS worldwide [26], our observations, if substantiated by randomized controlled studies, suggest far reaching public health implications.

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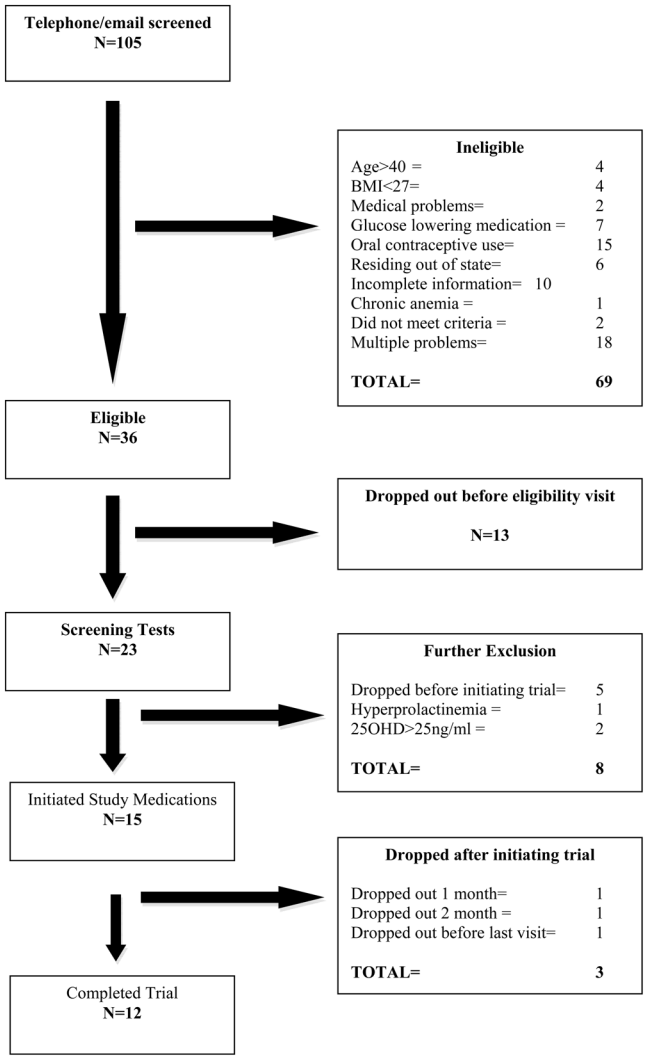

Figure 1.
Flow diagram outlines enrollment details for the clinical trial, registration number NCT00743574, *(clinicaltrials.gov).*

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Table I

Participant characteristics and biochemical data at baseline (B) and following completion (C) of 3 month supplementation with vitamin D and calcium.

Parameter	Baseline (n=12)	Completion (n=12)	P value
Age (years)	26.42 ± 6.11	-	
Waist Circumference (cm)	114.61 ± 20.90	114.55± 21.47	0.984[a]
BMI (kg/m^2)	39.84 ±10.86	39.83 ±10.53	0.982[a]
Blood Pressure (BP, mmHg)			
Systolic BP	120.67±16.11	113.5 ± 9.74	0.104[a]
Diastolic BP	76.17 ±10.65	70.83 ± 7.32	0.147[a]
Mean Arterial BP	99.71 ± 13.58	90.87 ± 10.18	0.101[a]
25OHD (ng/ml)	17.58 ± 4.56	28.758 ± 6.30	<0.001[a]
IFG[d] n(%)	5 (42)	7(58)	0.157[b]
IGT[e] n(%)	4 (33)	3 (25)	0.655[b]
Fasting Insulin (μIU/ml)	24.92 ± 15.42	25.17 ± 13.67	0.591[a]
Fasting Glucose (mg/dl)	101.67 ± 17.90	103.79 ± 22.83	0.386[a]
Fasting Glucose:Insulin Ratio[f]	5.57 ± 3.17	5. 50 ± 3.58	0.860[a]
HOMA[g]	6.68 ± 5.83	6.90 ± 5.74	0.736[a]
QUICKI[h]	0.13 ± 0.01	0.13 ±0.01	0.597[a]
AUC[i] Glucose (mg/min/120min)	16648.41± 3949.98	16726.56 ± 3941.80	0.915[a]
AUC[i] Insulin (μIU/ml/120min)	16136.50 ± 10086.29	16715.57 ± 10371.76	0.622[a]
Total Testosterone (ng/dl)	82 ± 44	72± 39	0.036[a]

Parameter	Baseline (n=12)	Completion (n=12)	P value
SHBG (nmol/L)	23.58 ±3.30	22.25± 2.20	0.718[a]
Androstenedione (ng/dl)	361 ± 204	299 ± 126	0.090[a]
FAI[j]	13.72 (4.68–26.96)	8.42 (4.86–25.88)	0.814[c]

Continuous data are presented as mean ± standard deviation if Gaussian in distribution, and as median (interquartile range if skewed); categorical data are presented as percentage.

[a] Student T test;

[b] McNemar test;

[c] Wilcoxon Sign Rank Test

[d] Fasting glucose ≥ 100 mg/dl

[e] 2 hour glucose>=140 & <200mg/dl

[f] G:I ratio<4.5 reflects insulin resistance [26]

[g] fasting glucose (mg/dl) x fasting insulin (mIU/ml)/405

[h] 1/log (fasting glucose (mg/dl) x fasting insulin (mIU/ml)

[i] AUC calculated by trapezoidal rule

[j] Free androgen index: total testosterone in nmol/L/SHBGin nmol/L X 100

Table II

Individual participant data at baseline (B) and following completion (C) of 3 month supplementation with vitamin D and calcium.

Subject	Age (Yrs)	Race[a]	Vitamin D Dose[b]	BMI (Kg/m^2)		25OHD (ng/dl)		MAP[c] (mmHg)		Glucose[d] (mg/dl)		Insulin [d] (μIU/ml)		OGTT		Total T[h] (ng/dl)		A[i] (ng/dl)	
				B	*C*	*B*	*C*	*B*	*C*	*B*	*C*	*B*	*C*	*B*	*C*	*B*	*C*	*B*	*C*
1	39	NHW	Low	56.6	55.1	18	19	91	100	104	116	30	40	IGT[e]	IGT[e]	27	25	90	78
2	28	H	Low	30.5	30.4	20	27	98	102	93	94	12	14	NL[f]	IGT[e]	90	55	516	288
3	23	H	Low	32.6	32.9	19	29	109	97	98	83	37	22	DM[g]	IGT[e]	138	111	307	252
4	29	NHW	Low	40.2	38.2	14	37	111	95	101	106	15	28	IGT[e]	NL[f]	36	37	236	290
5	23	NHW	Low	38.1	38.2	13	23	103	83	105	102	21	23	IGT[e]	NL[f]	45	39	230	229
6	23	H	High	43.2	43.9	23	25	97	97	94	95	36	26	NL[f]	NL[f]	132	105	269	362
7	23	NHW	High	62.1	60.7	22	43	72	90	155	170	61	55	DM[g]	DM[g]	40	40	182	243
8	37	NHW	High	41.1	43.1	16	30	117	70	95	100	8	7	NL[f]	NL[f]	31	28	142	165
9	21	NHW	High	28.2	27.5	12	30	90	76	92	104	11	12	NL[f]	NL[f]	82	84	547	408
10	26	H	High	45.5	46.7	10	25	112	99	90	88	33	34	NL[f]	NL[f]	114	104	501	283
11	26	NHW	High	28.5	28.1	20	28	83	85	102	101	11	11	IGT[g]	NL[f]	132	134	732	538
12	19	NHW	High	31.5	33.2	24	27	110	92	87	85	24	30	NL[f]	NL[f]	114	111	577	455

[a] NHW: Non Hispanic White; H: Hispanic

[b] Vitamin D2 dosing regimen: 50,000IU monthly (Low); 50,000IU weekly (High)

[c] Mean arterial blood pressure

[d] Fasting

[e] Impaired glucose tolerance evident during OGTT: 2 hour glucose>=140 & <200mg/d

[f] Normal glucose tolerance test: fasting glucose<100 and 2 hour glucose <140mg/dl

[g] Met OGTT criteria for diabetes based on 2 hour glucose level >=200mg/d

[h] Total testosterone

[i] Androstenedione

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