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RESEARCH ARTICLE

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# Comparison of the Effect of Oral versus Injectable Vitamin D on Serum Level of Vitamin D in Children with Vitamin D Deficiency Referred to the Taleghani Hospital in Gorgan

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**ABSTRACT**

**Introduction:** Vitamin D deficiency in children is associated with short stature, underdevelopment, respiratory infections, and many other features that increase the risk of childhood complications. Various studies have been conducted on different and appropriate strategies for the treatment of vitamin D deficiency that there are disagreements about the use of vitamin D3 or vitamin D2, oral versus intramuscular (IM), fixed or titrated doses, low daily doses or intermittent high doses. (17) This study also attempts to answer one of these cases and designed to compare the effect of vitamin D injectable form versus oral pearl vitamin D on serum vitamin D level in children with vitamin D deficiency referred to Taleghani Gorgan Training Center.

**Material and Methods:** In this study, one group was given 1 pearl vitamin D every week for eight weeks, and the second group was given a single dose of vitamin D 300,000 unit after 1 month and 3 months of treatment. Serum vitamin D levels were measured in all patients at Taleghani Gorgan Medical Center by one person and with one kit at all stages (baseline, 1 month, and 3 months after treatment).

**Results:** In this study, 118 patients were enrolled in the study, 5 in the oral treatment group and 4 in the injection treatment group were excluded due to lack of follow-up, discontinuation of medication and incomplete patient records. Finally, 54 patients were in the oral treatment group and 55 in the injection treatment group. The mean age of the patients was 8.21 years. The frequency distribution of patients in both groups was evaluated by gender. In the oral treatment group, 22 (40.7%) were male, and 15 (27.3%) were male. Which was not statistically significant (p = 0.138)the changes in vitamin D 4 weeks after treatment and 3 months after treatment did not differ by gender and body mass index. (p >0.05).Changes in vitamin D levels 4 weeks after treatment and 3 months after treatment in the oral and injection groups were evaluated by age (less than 8 years old / over 8 years old), which was statistically significant in the oral treatment group. (p >0.05).But in the injection treatment group, the increase in vitamin D after 4 weeks was significantly greater in the age of 8 and less compared to the age of greater than 8 years (27.60 vs. 20.85 and p = 0.048,). In Long-term, 3 months later, there was no difference. (p>0.05)

**Conclusion:** The results of the present study and previous studies show that both oral and injectable vitamin D therapy is equally effective in treating this deficiency in children. However, our study has shown that in younger children, injectable form in the short term yields a better response, but further studies with larger sample sizes are needed to generalize these results.

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## INTRODUCTION

Vitamin D plays a significant role in human health, survival, and fertility (1). Numerous studies have emphasized its role in the prevention of diseases such as heart disease, malignancies, inflammatory bowel diseases, multiple sclerosis, rheumatoid arthritis, type 1 diabetes, immune system diseases, and infectious diseases (2-4). Vitamin D increases the absorption of phosphorus and calcium from the intestines and reduces their excretion from the kidneys and strengthens the osteogenesis process. Therefore, its deficiency is one of the critical factors in the development of bone metabolism disorders (2).

Unfortunately, in most cases, the amount of vitamin D supplied by the body through food sources is insufficient, and, on the other hand, the nutrient-enriched with this vitamin is limited and unable to supply the needed amount of children and adults. This is the primary cause of the prevalence and epidemic of vitamin D deficiency, even in European and American countries. In fact, vitamin D production in the vicinity of the sun's ultraviolet radiation is the major source of its supply (5).

A reduction in serum 25-hydroxyvitamin D levels of less than 50 nmol/liter means vitamin D deficiency. Accordingly, serum levels of less than 25 nmol/liter are severely deficient, and levels of 25 to 75 nmol/liter are moderate vitamin D deficiency. (5-6)In one study in Europe, 97-93% of children in Denmark and Finland had vitamin D levels below 20 ng/ml (7) In a study in Iran, vitamin D deficiency was observed in 95.4% of children (8).

Vitamin D deficiency in children is associated with short stature, underdevelopment, respiratory infections, and many other features that increase the risk of childhood complications (10 - 9).Severe vitamin D deficiency, however, can impair mineralization and rickets in children and osteomalacia in adults. (10)Both forms of cholecalciferol (vitamin D3) and ergocalciferol (vitamin D2) are used to treat vitamin D deficiency. However, there is disagreement about the dose required to reach the optimum level of this vitamin as well as how to use (oral / injection) (11,12).

In a 2017 study by Gupta et al. in India, the efficacy of oral vitamin D treatment compared to intramuscular injection was evaluated, and the results showed that both oral and intramuscular injections were effective in treating vitamin D deficiency. But 25-hydroxyvitamin D levels in the IM group showed a steady and significant increase from baseline (13). In another study by Wylon et al., Conducted in Germany in 2015, the effect of a single high intramuscular dose was compared to long-term oral vitamin D supplementation. The results of this study showed that there was no significant difference in serum 25 (OH) D concentration after 28 days between the two methods of administration. (14-16)

Various studies have been conducted on different and appropriate strategies for the treatment of vitamin D deficiency that there are disagreements about the use of vitamin D3 or vitamin D2, oral versus intramuscular (IM), fixed or titrated doses, low daily doses or intermittent high doses. (17) This study also attempts to answer one of these cases and designed to compare the effect of vitamin D injectable form versus oral pearl vitamin D on serum vitamin D level in children with vitamin D deficiency referred to Taleghani, Gorgan Training Center.

## MATERIALS AND METHODS

This study was a clinical trial with no blinding. All children between the ages of 5-15 years referred to Taleghani Gorgan Medical Center were considered as the statistical population of the study. The sampling method was accessible. The family was recommended as one of the oral or injection modes of treatment. Treatment was initiated if parental consent was given based on the type of treatment recommended. After obtaining permission from the University Research Council and receiving the approval of the Ethics Committee and Proposal Registration in the Clinical Trial System, the researcher referred to the research unit.

### Patient selection and sampling

Tellioglu et al.'s (17) study results and appropriate statistical methods were used to determine the sample size. The sample size was calculated using 90% power and 5% alpha using GPower software. Finally, 118 patients (59 patients in each treatment group) were enrolled. Inclusion criteria included no kidney, liver, heart disease, and gastrointestinal disease leading to malabsorption. Having a vitamin D level of less than 20, not receiving vitamin D medication and other vitamin D deficiency treatments in the last six months.

Patients were excluded from the study with no follow-up and treatment because of death or family transfer to another city, drug side effects, and any vitamin D supplementation uses other than the treatment method of study.

Children in the age group of 5 to 5 years who have developmental impairment in their assessment and initial visit or parents who have been asked for a vitamin D level check-up were asked for a vitamin D test.

After receiving the test results, they were included in the study if their vitamin D level was lower than 20 ng/ml and had inclusion criteria.

Method of trial:

Parents were given the necessary explanations for the treatment. It was also emphasized that during the study period, using vitamin D compounds would be avoided.

If patients were in the injection group, they received 300000 U vitamin D single-dose intramuscular and One month and six months after the day of injection, the serum level of vitamin D was checked, and if the sample was in the oral treatment group, one pearl of vitamin D50000 was administered for eight weeks each week. One month and six months after the end of eight weeks of drug use, serum vitamin D levels were checked. A demographic questionnaire was completed for each sample. Designers and patients were aware of the assigned treatment group, and blinding was not feasible in this study. Serum vitamin D test was also performed for patients at Taleghani Gorgan Medical Center by one person in the morning and with one kit (baseline, 1, and 3 months after treatment). Ethical considerations were taken into account at all stages.

### Statistical analysis

The data were analyzed after coding and entering into SPSS 20 software. Mean, standard deviation, frequency, and percentage indices were used to describe the data. Independent t-test, the chi-square test was used to compare groups, and non-parametric equivalent tests were used if needed.

## RESULTS

In this study, 118 patients were enrolled in the study, 5 in the oral treatment group and 4 in the injection treatment group were excluded due to lack of follow-up, discontinuation of medication and incomplete patient records. Finally, 54 patients were in the oral treatment group and 55 in the injection treatment group. The mean age of the patients was 8.21 years, with a standard deviation of 2.06; the lowest age was 5 years, and the highest age was 12 years. It should be noted that no side effects were observed during the study.

The frequency distribution of patients in both groups was evaluated by gender. In the oral treatment group, 22 (40.7%) were male, and 15 (27.3%) were male, which was not statistically significant (p = 0.138). (Figure 1) The frequency distribution of patients' place of residence was evaluated in two groups, which was not statistically significant (p = 0.973). Also, the frequency distribution of parents' education in the two groups was not statistically significant, and there was no difference between the two groups (p >0.05).

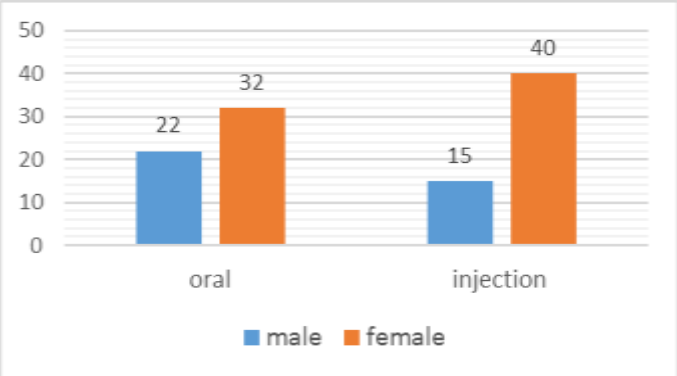


**Figure 1: Frequency distribution of gender in two groups**

Frequency distribution of vitamin A + D history in the first 2 years of life and vitamin D history in the past few months were studied in patients in both groups, which was not statistically significant. (P = 0.112) and (p = 0.150), respectively

Mean, standard deviation, minimum and maximum vitamin D levels before and after treatment, 4 weeks after treatment, 3 months after treatment were compared between the two groups using a nonparametric test. Although vitamin D levels were slightly higher in the oral treatment group at week 4 and month 3, no significant difference was found between the two groups in any of the three stages of vitamin D treatment (p >0.05). Also, mean, standard deviation, minimum and maximum changes of vitamin D levels at 4 weeks after treatment, and 3 months after treatment were evaluated in both groups, and There was no significant difference between the two groups (p >0.05).

**Table1: Mean, standard deviation, minimum and maximum changes in vitamin D levels 4 weeks after treatment and 3 months after treatment in both groups**

| p-value | Maximum | Minimum | Std. Deviation | Mean | تعداد | Vitamin D level | |
|---|---|---|---|---|---|---|---|
| 0.694 | 53.70 | 3.20 | 13.75655 | 23.5352 | 54 | Oral treatment | Changes in vitamin D levels 4 weeks after treatment |
| | 58.50 | 4.80 | 12.66765 | 24.5327 | 55 | Injection treatment | |
| | 58.50 | 3.20 | 13.16643 | 24.0385 | 109 | total | |
| 0.661 | 76.70 | 18.00 | 14.32776 | 43.9426 | 54 | Oral treatment | Changes in vitamin D levels 3 months after treatment |
| | 71.50 | 15.40 | 12.13316 | 42.8236 | 55 | Injection treatment | |
| | 76.70 | 15.40 | 13.21606 | 43.3780 | 109 | total | |

Man-Whitney

On the other hand, the changes in vitamin D 4 weeks after treatment and 3 months after treatment did not differ by gender and body mass index. (p >0.05).

Changes in vitamin D levels 4 weeks after treatment and 3 months after treatment in the oral and injection groups were evaluated by age (less than 8 years old / over 8 years old), which was statistically significant in the oral treatment group. (p >0.05).

But in the injection treatment group, the increase in vitamin D after 4 weeks was significantly greater in the age of 8 and less compared to the age of greater than 8 years (27.60 vs. 20.85 and p = 0.048,). In Long-term, 3 months later, there was no difference. (p>0.05)(table2)

**Table2: Mean, standard deviation, minimum and maximum changes in vitamin D levels 4 weeks after treatment and 3 months after treatment in the injection group according to the age of patients**

| p-value | Maximum | Minimum | Std. Deviation | Mean | N | Vitamin D level | |
|---|---|---|---|---|---|---|---|
| 0.048 | 58.50 | 7.00 | 13.56255 | 27.6000 | 30 | =>8 years old | Changes in vitamin D levels 4 weeks after treatment |
| | 36.50 | 4.80 | 10.62635 | 20.8520 | 25 | <8 years old | |
| | 58.50 | 4.80 | 12.66765 | 24.5327 | 55 | total | |
| 0.797 | 71.50 | 15.40 | 13.85433 | 43.2133 | 30 | =>8 years old | Changes in vitamin D levels 3 months after treatment |
| | 64.00 | 27.80 | 9.94397 | 42.3560 | 25 | <8 years old | |
| | 71.50 | 15.40 | 12.13316 | 42.8236 | 55 | total | |

Man-Whitney

## DISCUSSION

The aim of the present study was to compare the effect of vitamin D injectable form with oral treatment on serum level in children with vitamin D deficiency referred to Taleghani Gorgan Medical Center. In the present study, 54 patients were in the oral treatment group and 55 in the injection treatment group. The mean age of the patients was 8.21 years. Frequency of sex, place of residence, history of vitamin A + D use in the first 2 years of life, history of vitamin D use in the last few months, and parental education of patients in both The study group was evaluated, which was not statistically significant.

In this study, in spite of slightly higher levels of vitamin D in the treatment group at week 4 and month 3, no significant differences were found between the two groups in any of the three stages of vitamin D level evaluation.

Mean, standard deviation, minimum and maximum changes in vitamin D levels were evaluated 4 weeks after treatment and 3 months after treatment in both groups which There was no significant difference in vitamin D levels between the two groups. In addition, vitamin D changes in both groups were analyzed by gender and body mass index, which was not statistically significant.

In our study in the injection treatment group, the increase in vitamin D after 4 weeks was significantly higher at the age of 8 and less

compared to the age of greater than 8 years (27.60 vs. 20.85 and p = 0.048,). In Long-term, 3 months later, there was no difference.

Studies have been performed in this area, for example, in the study of Wylon et al. (16) evaluated single-dose intramuscular pharmacokinetics with long-term oral vitamin D supplementation. The results of this study showed that there was no significant difference in serum 25 (OH) D concentration after 28 days between the two methods of administration. This results confirm the results of our study and indicate that both methods are useful in the treatment of vitamin D deficiency in another study that was consistent with the results of our study.

In the study of Mondal et al. (15), the effect of 600000 IU intramuscular single dose of vitamin D was compared with oral dose of vitamin D. The results of this study showed that there was no significant difference in the efficacy of the two regimens base on the biochemical and radiological parameters. Finally, it was concluded that oral or once intramuscular injections of 600,000 IU of vitamin D were effective and safe in the treatment of nutritional rickets (15).

In our study, the results showed that in low ages (less than 8 years), the intramuscular injection was associated with better response in the short term.

In this regard, the study of Rabea et al. (14) evaluated the effect of oral and injectable vitamin D supplementation treatment in patients with rickets.

The results of this study showed that patients who received intramuscular doses responded rapidly to treatment, whereas infants who received oral doses had less response (14). This confirms the results of our study, with the exception that this study was performed only on infants and had a smaller sample size than ours, but our study included a broader age range. It should be noted that no side effects were observed during our study.

In a review study by Mazari et al. (19), the efficacy and response of oral vitamin D treatment and intramuscular injection to the treatment of vitamin D deficiency in rickets were compared.

The results of this study showed that no adverse side effects were observed in either group of children treated with the oral or injectable regime and both oral and injectable forms were tolerable. The cost of oral and injectable vitamin D was approximately the same (19) which confirms the results of our study.

On the other hand, studies have concluded the superiority of one method over another, for example in the study of Gupta et al. (13), the effect of oral vitamin D compared with intramuscular injection form of vitamin D. In this study, 40 individuals with vitamin D deficiency were divided into two groups. Both oral and intramuscular injections are effective in treating vitamin D deficiency, but 25-hydroxyvitamin D levels in the IM cholecalciferol group showed a significant increase from baseline (13). Differences between studies may be due to differences in the method of study, drug dosage, age group, and sample size, which requires further studies with larger sample size.

## CONCLUSION

The results of the present study and previous studies show that both oral and injectable vitamin D therapy is equally effective in treating this deficiency in children. However, our study has shown that in younger children, injectable form in the short term yields a better response, but further studies with larger sample sizes are needed to generalize these results.

## REFERENCES


1. Hagenau T, VR, Gissel TN, Poulsen CS, Erlandsen M, Mosekilde L, Vestergaard P, Global vitamin D levels in relation to age, gender, skin pigmentation and latitude: an ecologic meta-regression analysis. OsteoporosInt 2009; 20(1): p. 133-40.
2. Holick MF, Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. Am J Clin Nutr 2004; 79(3): p. 362-71.
3. Hernan, MA, MJ. Olek, and A. Ascherio, Geographic variation of MS incidence in two prospective studies of US women. Neurology 1999; 53(8): p. 1711-8.
4. MF, H, Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. Am J Clin Nutr 2004; 80: p. 11.
5. Holick, M, Vitamin D deficiency: a worldwide problem with health consequences. American Journal of Clinical Nutrition 2008; 87: p. 7.
6. Stumpf WE, Sar M, Reid FA, et al. Target cells for 1, 25-dihydroxyvitamin D in intestinal tract, stomach, kidney, skin, pituitary, and parathyroid. Science 1979; 206:1188
7. Andersen R, Mølgaard C, Skovgaard LT, Brot C, Cashman KD, Chabros E, et al. Teenage girls and elderly women living in northern Europe have low winter vitamin D status. Eur J Clin Nutr 2005; 59(4): 533-41.
8. Namakin K, Zardast M, Sharifzadeh G, Azarkar Z. Prevalence of vitamin D deficiency in 7- 11 year old children in Birjand, east of Iran, 2012. Iran J Pediatr 2013; 23 Suppl 1: 8.
9. Marwaha RK, Tandon N, Agarwal N, Puri S, Agarwal R, Singh S, et al. Impact of two regimens of Vitamin D supplementation on

calcium: vitamin D-PTH axis of schoolgirls of Delhi. Indian Pediatr. 2010;47:761-9.
10. Arya V, Bhambri R, Godbole M, Mithal A. Vitamin D status and its relationship with bone mineral density in healthy Asian Indians. Osteoporos Int. 2004;15:56-61.
11. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of Vitamin D deficiency: An Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011;96:1911-30.
12. Goswami R, Gupta N, Ray D, Singh N, Tomar N. Pattern of 25-hydroxyvitamin D response at short (2 month) and long (1 year) interval after 8 weeks of oral supplementation with cholecalciferol in Asian Indians with chronic hypovitaminosis D. Br J Nutr 2008;100:526-9.
13. Gupta N, Farooqui KJ, Batra CM, Marwaha RK, Mithal A. Effect of oral versus intramuscular Vitamin D replacement in apparently healthy adults with Vitamin D deficiency. Indian J Endocr Metab 2017;21:131-6.
14. Hoda Rabea, Mohamed E. Abdelrahim , Mohamed H. Meabed. The efficacy of oral versus parentral vitamin D in treatment of nutritional. Medicine Science 2012;1(4):244-53.
15. Krishanu Mondal, Anju Seth, Raman K. Marwaha, Dinesh Dhanwal,3Satinder Aneja, Ritu Singh, Pitambar Sonkar. A Randomized Controlled Trial on Safety and Efficacy of Single Intramuscular versus Staggered Oral Dose of 600 000IU Vitamin D in Treatment of Nutritional Rickets. Journal of Tropical Pediatrics Vol. 60, No. 3.203-210.
16. Katharina Wylon, Gennadiy Drozdenko, Alexander Krannich, Guido Heine, Sabine DoÈ lle, Margitta Worm. Pharmacokinetic Evaluation of a Single Intramuscular High Dose versus an Oral Long- Term Supplementation of Cholecalciferol. PLoS ONE 12(1): e0169620.
17. Tellioglu A, Basaran S, Guzel R, Seydaoglu G. Efficacy and safety of high dose intramuscular or oral cholecalciferol in vitamin D deficient/insufficient elderly. Maturitas. 2012 Aug 1;72(4):332-8.
18. Billoo, A Gaffar & Murtaza, Ghulam & Memon, Ashraf & Ahmed Khaskheli, Sultan & Iqbal, Khalid & Rao, Masood. (2009). Comparison of oral versus injectable vitamin-D for the treatment of nutritional vitamin-D deficiency rickets. Journal of the College of Physicians and Surgeons--Pakistan : JCPSP. 19. 428-31.
19. Mazari (2017) Comparision of Response of Oral Versus Injectible Vitamin D in Children Having Rickets. Vitam Miner 6: 165. doi:10.4172/2376-1318.1000165
20. Marwaha RK, Tandon N, Reddy DR, Aggarwal R, Singh R, Sawhney RC, et al. Vitamin D and bone mineral density status of healthy schoolchildren in Northern India. Am J Clin Nutr 2005; 82:477-82.
21. Harinarayan CV. Prevalence of Vitamin D insufficiency in postmenopausal South Indian women. Osteoporos Int 2005; 16:397-402.
22. Sahu M, Bhatia V, Aggarwal A, Rawat V, Saxena P, Pandey A, et al. Vitamin D deficiency in rural girls and pregnant women despite abundant Sunshine in Northern India. Clin Endocrinol (Oxf) 2009; 70:680-4.
23. Zargar AH, Ahmad S, Masoodi SR, Wani AI, Bashir MI, Laway BA, et al. Vitamin D status in apparently healthy adults in Kashmir Valley of Indian subcontinent. Postgrad Med J 2007; 83:713-6.
24. Harinarayan CV, Ramalakshmi T, and Prasad UV, Sudhakar D. Vitamin D status in Andhra Pradesh: A population based study. Indian J Med Res 2008; 127:211-8.
25. Ritu G, Gupta A. Vitamin D deficiency in India: Prevalence, causalities and interventions. Nutrients 2014; 6:729-75.
26. Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. Am J Clin Nutr 2006; 84:18-28.
27. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of Vitamin D deficiency: An Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011; 96:1911-30.
28. Goswami R, Gupta N, Ray D, Singh N, Tomar N. Pattern of 25-hydroxyvitamin D response at short (2 month) and long (1 year) interval after 8 weeks of oral supplementation with cholecalciferol in Asian Indians with chronic hypovitaminosis D. Br J Nutr 2008;100:526-9.
29. Francis R, Aspray T, Fraser W, Gittoes N, Javaid K. Vitamin D and Bone Health: A Practical Clinical Guideline for Patient Management. The National Osteoporosis Society. Camerton, Bath; 2013. Available from: https://www.nos.org.uk/document.doc?id=1352. [Last accessed on 2015 Dec 10].
30. Heaney RP, Davies KM, Chen TC, Holick MF, Barger-Lux MJ. Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol. Am J Clin Nutr 2003; 77:204-10.
31. Whyte MP, Haddad JG Jr., Walters DD, Stamp TC. Vitamin D bioavailability: Serum 25-hydroxyvitamin D levels in man after oral, subcutaneous, intramuscular, and

intravenous Vitamin D administration. J Clin Endocrinol Metab 1979; 48:906-11.
32. Mawer EB, Backhouse J, Holman CA, Lumb GA, Stanbury SW. The distribution and storage of Vitamin D and its metabolites in human tissues. Clin Sci 1972; 43:413-31.
33. Cipriani C, Romagnoli E, Pepe J, Russo S, Carlucci L, Piemonte S, et al. Long-term bioavailability after a single oral or intramuscular administration of 600,000 IU of ergocalciferol or cholecalciferol: Implications for treatment and prophylaxis. J Clin Endocrinol Metab 2013; 98:2709-15