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Effect of Aminoacids Arginine and Lysine on Osteoblastic Activity.

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Abstract

Background:

Osteoblastic activity is an indicator of bone formation and remodelling.Bone formation plays a vital role in fracture healing and implant osseointegration. Several aminoacids have therapeutic role of which the rationale for using lysine is the stimulation of instestinal calcium absorption and renal conservation and of the cross linking process of bone collagen.As far as Arginine is concerned ,it is involved in the synthesis of polyamine and L-Proline(substrate for collagen formation),of growth hormone and insulin like growth factor and NO production. The effectiveness of these aminoacids have been shown individually in several studies.Yet pooling of evidence regarding influence of aminoacids on osteoblasts has not been documented in literature so far.

Aim: To evaluate the effectiveness of L-Arginine and L-Lysine on osteoblastic activity.

Materials and Methods: Relevant articles were selected by searching the following databases:
Pubmed, & LILACS , Metapress Database. Hand search was also carried out until June 2015.

Results: four studies were included in this review, of which 2 studies were conducted on rat osteoblastic cultures, one study was conducted on Human osteoblastic cultures and the other study in human osteopenic osteoblastic cultures. The included articles would then be subjected to data extraction and subsequent statistical analysis.

Conclusion: Evidence from the included articles suggests simultaneous administration of aminoacids lysine and arginine showed significant osteoblastic activity compared to arginine and lysine when administered individually .

Null Hypothesis

Null hypothesis formulated for the review was, L-Lysine and L-Arginine doesnot have a positive effect on osteoblastic activity and in turn bone formation.

Alternate Hypothesis

Alternate hypothesis formulated for the review was, L-Lysine and L-Arginine have a positive effect on osteoblastic activity and in turn bone formation.

INTRODUCTION:

Osteoblasts has been defined as cells that produce collagen needed for the development of new bone[(1)].

Several factors such as age, hormonal status, diet are implicated in the pathogenensis of osteoporosis, disease charecterised by progressive bone loss[(2)].. Several studies showed that a relationship exists between bone health and nutrition in all age categories, especially in elderly population[(4-6)]. Low protein intake induceses a decrease in bone density of femoral neck[(7)], while protein supplementation improves the medical outcome of hip fracture patients[(8)]. Recently Munger et all[(10)] reported that protein from animal sources appears to protect against fractures, while proteins from vegetable sources has no effect, since vegetable proteins have low levels of essential aminoacids. In order to better clarify the role of some essential aminoacids in bone health, growth, alkaline phosphatase activity, and collagen sysnthesis were evaluated in osteoblast cultures obtained from calvaria of newborn Sprague – dawley rats and incubated with lysine, threonine, methionine, tryptophane and arginine by Maria Teresa Conconi et all, showed that the essential aminoacids can stimulate bone formation and could represent useful agents for prevention and therapy of osteoporosis.

Structured Question

1. Do aminoacids like arginine and lysine have an effect on osteoblastic activity?

PICO Analysis

P - osteoblasts

Intervention with arginine and lysine..

Outcome whether there is an increase in osteoblastic activity following use of aminoacids using different assessment techniques such as biochemical and gene expression, Quantitative ALP,NO,OC,MTT,Collagen type 1,Cell count calculation.

MATERIALS A D METHOD

Search sources:

PUB MED,
LILACS databases
HAND SEARCH

Search methodology:

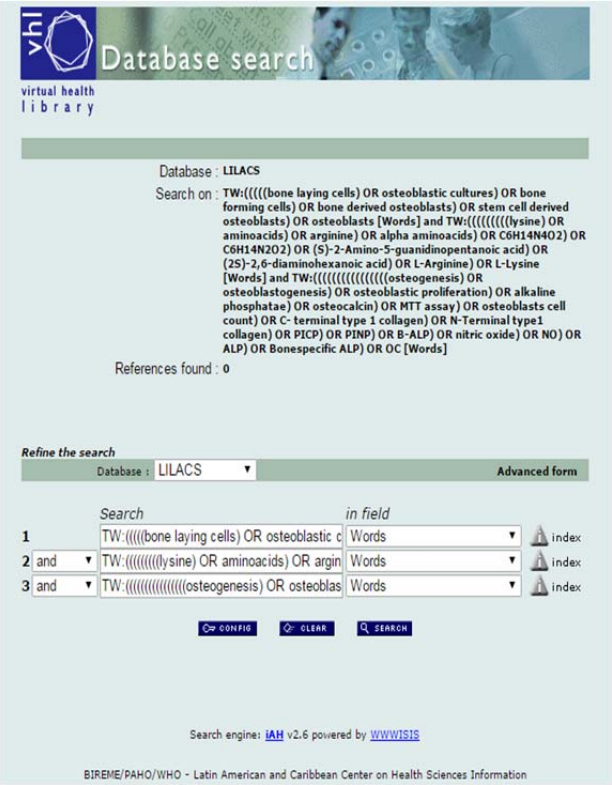
A systematic search strategy of English literature on the In the intial phase of the review, a computerized literature search for studies which tested the effectiveness of L-Lysine and L-Arginine on osteoblastic activity was performed in the above mentioned sources of databases till June 2015

In addition an hand search was carried out in Indian journal of orthopaedics

The search was performed using keywords and terms that are mentioned in Table 1.
No Limits and language restriction were applied during the electronic search to include all the potentially relevant articles in the systematic review. Further the reference list of reviews and the selected articles were checked for possible additional studies.

Inclusion criteria:
The search was then narrowed down manually by the reviewer according to the inclusion criteria of the present systematic review to include
-in situ studies.
-Studies evaluating effect of arginine and lysine on osteoblastic activity
-Studies comparing arginine and lysine with other aminoacids.
- cellular studies.

Exclusion criteria:
The exclusion criteria for the present study contained
- aminoacids on substrates
- amino acid peptides



SELECTION FLOW CHART:

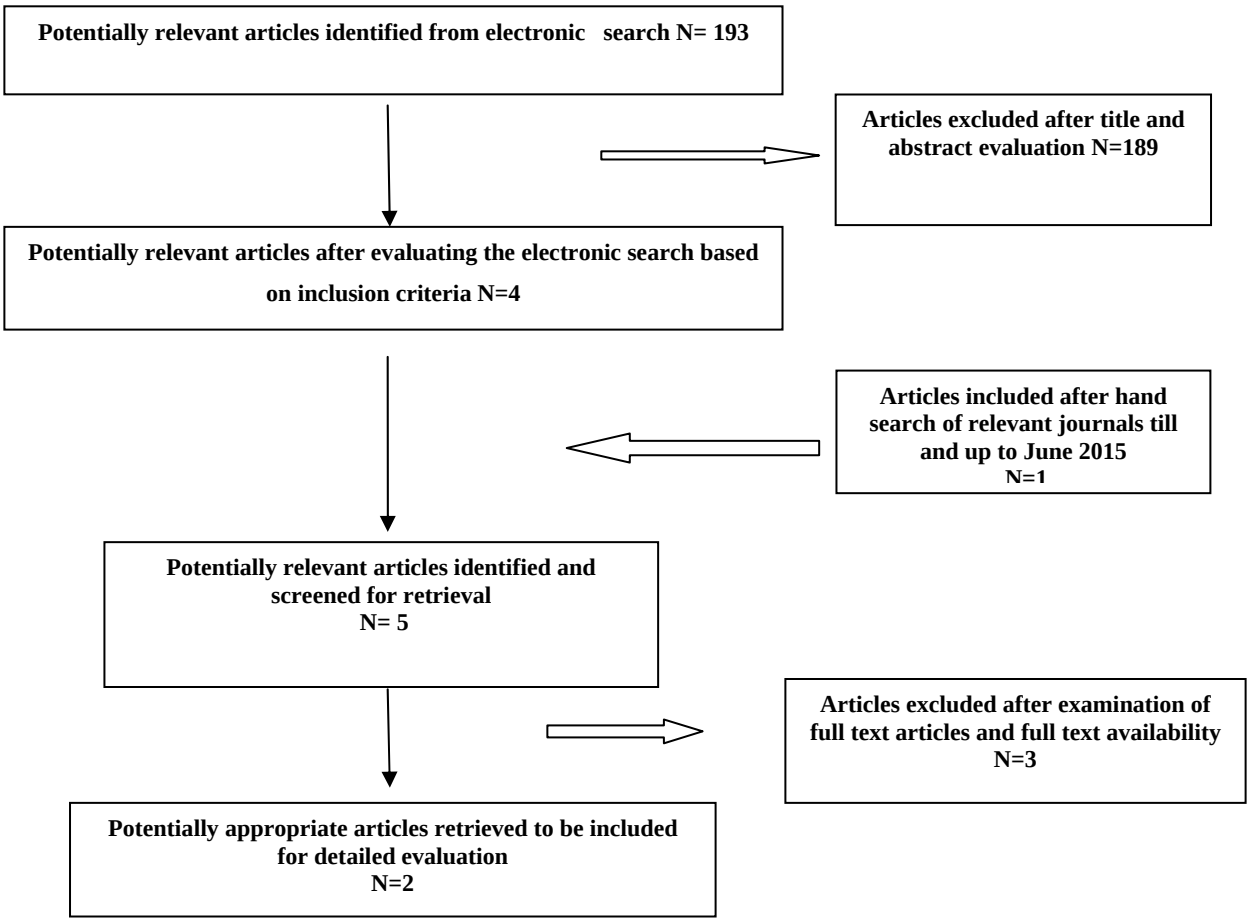


Table 1: Search Strategy

Search	Add to builder	Query	Items found
#37	Add	Search (((((((bone laying cells) OR osteoblastic cultures) OR bone forming cells) OR bone derived osteoblasts) OR stem cell derived osteoblasts) OR osteoblasts)) AND ((((((((((lysine) OR aminoacids) OR arginine) OR alpha aminoacids) OR C6H14N4O2) OR C6H14N2O2) OR (S)-2-Amino-5-guanidinopentanoic acid) OR (2S)-2,6-diaminohexanoic acid) OR L-Arginine) OR L-Lysine)) AND ((((((((((((((osteogenesis) OR osteoblastogenesis) OR osteoblastic proliferation) OR alkaline phosphatae) OR osteocalcin) OR MTT assay) OR osteoblasts cell count) OR C- terminal type 1 collagen) OR N-Terminal type1 collagen) OR PICP) OR PINP) OR B-ALP) OR nitric oxide) OR NO) OR ALP) OR Bonespecific ALP) OR OC)	193
#36	Add	Search ((((((((((((((osteogenesis) OR osteoblastogenesis) OR osteoblastic proliferation) OR alkaline phosphatae) OR osteocalcin) OR MTT assay) OR osteoblasts cell count) OR C- terminal type 1 collagen) OR N-Terminal type1 collagen) OR PICP) OR PINP) OR B-ALP) OR nitric oxide) OR NO) OR ALP) OR Bonespecific ALP) OR OC	238155
#35	Add	Search (((((((((lysine) OR aminoacids) OR arginine) OR alpha aminoacids) OR C6H14N4O2) OR C6H14N2O2) OR (S)-2-Amino-5-guanidinopentanoic acid) OR (2S)-2,6-diaminohexanoic acid) OR L-Arginine) OR L-Lysine	171705
#34	Add	Search (((((bone laying cells) OR osteoblastic cultures) OR bone forming cells) OR bone derived osteoblasts) OR stem cell derived osteoblasts) OR osteoblasts	48276
#33	Add	Search ALP	13597
#32	Add	Search NO	0
#31	Add	Search OC	15696
#30	Add	Search Bonespecific ALP	1
#29	Add	Search B-ALP	165
#28	Add	Search PINP	649
#27	Add	Search PICP	1146
#26	Add	Search N-Terminal type1 collagen	1
#25	Add	Search C- terminal type 1 collagen	1069
#24	Add	Search nitric oxide	139747
#23	Add	Search osteoblasts cell count	940
#22	Add	Search MTT assay	26918
#21	Add	Search osteocalcin	14659
#20	Add	Search alkaline phosphatase	1
#19	Add	Search osteoblastic proliferation	2739
#18	Add	Search osteoblastogenesis	750
#17	Add	Search osteogenesis	33716
#16	Add	Search L-Lysine	73533
#15	Add	Search L-Arginine	109475
#14	Add	Search (2S)-2,6-diaminohexanoic acid	0
#13	Add	Search (S)-2-Amino-5-guanidinopentanoic acid	1
#12	Add	Search C6H14N2O2	2
#11	Add	Search C6H14N4O2	3
#10	Add	Search alpha aminoacids	86861
#9	Add	Search aminoacids	845792
#8	Add	Search arginine	109475

Table 1: Search Strategy

Search	Add to builder	Query	Items found
#7	Add	Search lysine	73533
#6	Add	Search stem cell derived osteoblasts	1988
#5	Add	Search bone derived osteoblasts	4315
#4	Add	Search bone forming cells	15729
#3	Add	Search osteoblastic cultures	3124
#2	Add	Search bone laying cells	118
#1	Add	Search osteoblasts	33589

Table2 :variables of interest:

1	ALP levels
2	NO
3	OC
4	MTT
5	Collagen type 1
6	Cell count

Table 3 General Characteristics Of The Studies

Article	Study groups	Method of evaluation	Outcome	Limitations/ Future Scope
L-Arginine and L-Lysine stimulation on cultured human osteoblasts. P.Toricelli et al	arginine group : (n=4) lysine group: (n=4) arginine + lysine group: (n=4) Control group : (n=4) (N=16)	Cell growth and biochemical test of osteoblast metabolism.	Difference in the MTT,ALP,NO,Ca,P,OC,PICP readings within the groups after aminoacid treatment.	Effect of Lysine and Arginine on osteoblast proliferation and synthetic activity may be a beneficial support in prevention and treatment of different human bone pathology. Invitro study.
Effect of L-Lysine and L-Arginine on primary osteoblastic cultures from normal and osteopenic rats.M.Fini et al	arginine group : lysine group: arginine + lysine group: Control group : (N=10)	Biochemical test of osteoblast activity and gene expression at 48hrs and 7 days in normal and osteopenic osteoblsts	Difference in the MTT,ALP,NO,Ca,P,OC,PICP readings within the groups after aminoacid treatment.	Invitro study. New invitro and invivo investigations are required to acquire more details on the therapeutic effects of aminoacids and the mechanism behind them.
Essential amino acids increase the growth and alkaline phosphatase activity in osteoblasts cultured in vitro.	Arginine group : (n=4) Lysine group: (n=4) Tryptophan group: (n=4) Threonine group : (n=4) Methinine group : (n=4)	Biochemical test of osteoblast activity and gene expression at 48hrs and 72hrs in normal osteoblasts	Difference in the MTT,ALP and collagen synthesis readings within the groups after aminoacid treatment.	Invitro study
Human osteopenic bone-derived osteoblasts: essential amino acidstreatment effects. Torricelli P et al	arginine group : (n=6) lysine group: (n=6) arginine + lysine group: (n=6) Control group : (n=6) (N=24)	Cell growth and biochemical test of osteoblast metabolism.	Difference in the MTT,ALP,NO,Ca,P,OC,PICP readings within the groups after aminoacid treatment.	In vitro study

Table 4: Data Extraction Table

Sl no	Article	Author and Journal	Study Design	Sample Size	Participants and Group	Methodology	Parameters	Statistical Analysis	Results
1	L-Arginine and L-Lysine stimulation on cultured human osteoblasts.	P.Torricelli, M.Fini, G.Giavaresi, S.Gnudi, A.Nicolini, A.Capri. Biomedicine and Pharmacotherapy 56(2002)492-497	In vitro study	Sample size 16 culture chambers	16 culture chambers of human osteoblasts. arginine group : (n=4) lysine group: (n=4) arginine + lysine group: (n=4) Control group : (n=4) (N=16)	Human osteoblasts sterilely isolatedfrom small specimens of trabecular bone deived from the femoral head of patients who had undergone arthroplasty after traumatic fracture of femoral neck. Trabecular bone fragments were seeded into DMEM:F12 serum free culture medium and processed to obtain primary osteoblastic cultures. The cell cultures were treated with aminoacids as follows : Controls:sham treated; Four cultures treated daily with 0.625mg/ml Arginine;four cultures treated daily with 0.587mg/mlperday lysine and the remaining four treated with argine and lysine daily. The cultures were maintained in same condition for 7 days. End of 7 days supernatant of all cultures were centrifuged to remove particulate and immediately biochemical tests were performed .	ALP,MTT,NO,Ca, P,OC,PICP were the parameters of interest.	Students t test Oneway ANOVA Scheffes post hoc multiple comparison tests. P value was considerd significant at P < 0·05	**Mean ALP reading of different cultures:** Control Group:42.9±1.6 arginine Group: 50.5±2.1 lysine group:44.9±0.8 arginine plus lysine group:49.2±2.5 **Mean MTT in different cultures(optical density at 550nm):** Control group :0.65± 0.02 Arginine group:0.65±0.02 Lysine group:0.75±0.03 Arginine plus lysinegroup:0.66±0.02 **Mean OC(ng/ml) in different cultures:** Control group :37.0±0.9 Arginine group:41.3±1.7 Lysine group:37.1±3.5 Arginine plus lysine group:40.0±4.2 **Mean NO(µM) in different cultures:** Control group :3.3±0.3 Arginine group:3.5±0.2 Lysine group:2.8±0.2 Arginine plus lysine group:3.9±0.2 **Mean Ca(mg/dl) in different cultures:** Control group :4.9±0.6 Arginine group:4.9±0.3L ysine group:4.4±0.2 Arginine plus lysine group:4.3±0.2 **Mean P(mg/dl) in different cultures:** Control group :2.3±0.4 Arginine group:2.3±0.3 Lysine group:2.2±0.2 Arginine plus lysine group:2.1±0.1 **Mean PICP(ng/ml) in different cultures:** Control group :30.0±1.2 arginine group : 32.5±1.7 lysine group: 37.3±0.3 arginine + lysine group:28.5±0.6

Sl no	Article	Author and Journal	Study Design	Sample Size	Participants and Group	Methodology	Parameters	Statistical Analysis	Results
2	Effect of L-Lysine and L-Arginine on primary osteoblastic cultures from normal and osteopenic rats.M.Fini et al	M.Fini,P.Torricelli, G.Giaveresi,A.carpi, A.Nicolini,R.Giardino.	In vitro study	Sample size calculation is not mentioned	arginine group : lysine group: arginine + lysine group: Control group : (N=10)	osteoblasts sterilely isolatedfrom small trabeccular specimens derived fromright distal femurs of two different groups of rats aged 13 months: one group healthy and other group overectomised rats who have undergone bilateral overectomy 3 months before Trabecular bone fragments were seeded into DMEM:F12 serum free culture medium and processed to obtain 2 groups of primary osteoblastic – normal bone derived and osteopenic bone derived osteoblastic cultures. The cell cultures were treated with aminoacids as follows : Controls:sham treated; Four cultures treated daily with 0.625mg/ml Arginine;four cultures treated daily with 0.587mg/mlperday lysine and the remaining four treated with argine and lysine daily. The cultures were maintained in same condition for 7 days. End of 7 days supernatant of all cultures were centrifuged to remove particulate and immediately biochemical tests and gene expression tests were performed	ALP,NO,OC,MTT,Cell count,Collagen type 1	Scheffes multiple comparison test P value was considered significant at $P < 0{\cdot}05$	**Normal** **Bone derived osteoblasts:** **Mean ALP reading of different cultures:** Control Group:12.23±2.56 lys Group:11.96±1.30 argininegroup:12.73±1.44 arginine plus lysine group: **13.51±1.42** **Mean NO(µM) in different cultures:** **control group:** 4.71±0.23 arginine group : 4.79±0.22 lysine group: 4.40±0.32 arginine + lysine group:5.22±0.26 **Mean MTT in different cultures(optical density at 550nm):** Cont grp:1.26±0.09 arginine group :1.26±0.03 lysine group:1.20±0.15 arginine + lysine group:**1.38±0.17** **Mean OC(ng/ml) in different cultures:** **Control grp:15.23±1.27** arginine group : 14.23±0.18 lysine group:15.83±1.50 arginine + lysine group:**14.97±0.60** **Osteopenic bone derived osteoblasts** **Mean ALP reading of different cultures:** **Control grp:12.79±3.60** arginine group : 12.36±0.80 lysine group: 13.58±1.90 arginine + lysine group:12.76±0.96 **Mean NO(µM) in different cultures:** Control group:4.48±0.40 arginine group : 5.32±0.33 lysine group:4.26±0.19 arginine + lysine group:5.54±0.22 **Mean OC(ng/ml) in different cultures:** Control group:16.60±3.45 arginine group :13.53±0.60 lysine group: 14.20±1.51 arginine + lysine group:**15.63±.21** **Mean MTT in different cultures(optical density at 550nm):** Control group:1.60±0.27 arginine group :1.57±0.35 lysine group:1.55±0.35 arginine + lysine group:1.61±0.37

Sl no	Article	Author and Journal	Study Design	Sample Size	Participants and Group	Methodology	Parameters	Statistical Analysis	Results
3	Human osteopenic bone derived osteoblasts:essential aminoacid treatment. P.Torricelli et al. Artificial cells blood substitutes and immobile biotech 2003 Feb 31(1) 35-46.	M.Fini,P.Torricelli, .Giaveresi,A.carpi, A.Nicolini,R.Giardino	In vitro culture studies. **EVIDENCE BASED MEDICINE LEVELS of Evidence:** 5	Sample size 4 x(24 well plates) =96wells in 4 plates of human osteopenic bone culture from 76±6 years old female patients who underwent arthroplasty for traumatic femur neck fracture.	participants N = 96 wells Control group :6 Arginine group:6 Lysine group:6 Arginine plus lysine group:6	Human osteopenic bone derived from osteoblasts sterilely isolatedfrom small specimens of trabecular bone derived from the femoral head of 78±6 year old women patients who had undergone arthroplasty after traumatic fracture of femoral neck. Trabecular bone fragments were seeded into DMEM:F12 serum free culture medium and processed to obtain primary osteoblastic cultures. The cell cultures were treated with aminoacids as follows : Controls:sham treated; Four cultures treated daily with 0.625mg/ml Arginine;four cultures treated daily with 0.587mg/mlperday lysine and the remaining four treated with argine and lysine daily. The cultures were maintained in same condition for 7 days. End of 7 days supernatant of all cultures were centrifuged to remove particulate and immediately biochemical tests were performed	ALP,MTT,NO,Ca, P,OC were the parameters of interest.	SPSS PC+ Students independent t test , Oneway ANOVA Scheffes post hoc multiple comparison tests. P value was considerd significant at $P < 0{\cdot}05$	**Mean ALP in different cultures in IU/L:** Control group :41.5±1.9 Arginine group:53.9±4.0 Lysine group:47.0±1.2 Arginine plus lysine group:54.9±1.0 **Mean MTT in different cultures(optical density at 550nm):** Control group :0,53±0.03 Arginine group:0.46±0.01 Lysine group:0.57±0.01 Arginine plus lysinegroup:0.55±0.2. **Mean OC(ng/ml) in different cultures:** Control group :36.8±1.0 Arginine group:38.0±1.2 Lysine group:37.0±0.6 Arginine plus lysine group:38.5±1.7 **Mean NO(µM) in different cultures:** Control group :3.6±0.1 Arginine group:3.9±0.1 Lysine group:2.7±0.3 Arginine plus lysine group:3.6±0.1 **Mean Ca(mg/dl) in different cultures:** Control group :5.1±0.4 Arginine group:4.9±0.4 Lysine group:5.7±0.2 Arginine plus lysine group:5.0±0.2 **Mean P(mg/dl) in different cultures:** Control group :2,5±0.1 Arginine group:2.2±0.1 Lysine group:2.8±0.3 Arginine plus lysine group:2.2±0.1

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Table 5 Level of Evidence Table (Based on Centre for Evidence-Based Medicine, Oxford)

Sl no	Author & Year	Study Design	Level of Evidence
1	M.Fini et al 2001	In vitro	5
2	P.Torricelli et al 2002	In vitro	5
3	P.Torricelli etal 2003	In vitro	5

1a: Systematic review of trials displaying worrisome heterogeneity

Table 6: List Of Excluded Articles After Full Text Analysis:

Article	Reason for exclusion
1.Effect of amino acids lysine and arginine on fracture healing in rabbits:A radiological and histomorphological analysis. Journal of Orthopaedics,2009 Oct-Dec;43(4):328-334. Shivam sinha and Satish Chandra Goel	Out comes reported were radiographic grading 0,1,2,3
2. Essential amino acids increase the growth and alkaline phosphatase activity in osteoblasts cultured in vitro.IL Farmaco 56 (2001) 755-761	Outcome Data reporting incomplete.
3. Human osteopenic bone-derived osteoblasts: essential amino acidstreatment effects.Torricelli P et al	Full text not available

RESULTS:

Simultaneous administration of aminoacids lysine and arginine showed significant osteoblastic activity compared to arginine and lysine when administered individually.

DISCUSSION:

In 2001 Maria Teresa Conconi et all, showed that the essential aminoacids can stimulate bone formation and could represent useful agents for prevention and therapy of osteoporosis.

In 2001, M.Fini et all described that the potential effect of lysine and arginine on bone could be related, atleast in part, to an improvement in NO production and type 1 collagen synthesis by osteoblast both in normal and osteopenic bone. In osteopenic derived osteoblasts the synthetic phase was preceeded by an initial increase in cell proliferation

In 2002, P.Torricelli et all demonstrated the effects of argenine and lysine on human osteoblastic cells. Argenine administration significantly increased ALP, NO, PICP and IGF-I production and reduce the level of IL-6. Lysine administration over the same time interval mainly affected cell proliferation, as evidenced by MTT test and immune staining for PDGF. The same positive evidence of single administrations of the two aminoacids resulted from their simultaneous administration. However, synergism could be demonstrated as decrease in the level of IL-6. Argenine and Lysine show a positive effect on human osteoblasts, which is related partly to the production of factors required for matrix synthesis, and partly to the direct or mediated activation of cell proliferation.

In 2003 Torricelli et al used human osteopenic bone derived osteoblasts to study the effects of aminoacids arginine and lysine on osteoblasts.their results proved that arginine and lysine stimulation has a positive effect on osteoblast proliferation,activation and differensiation.Therefore administration of these aminoacids may aid in bone formation and in treating pathologies like osteoporosis.

Future randomized controlled studies are required for comparison of the effectiveness of these treatment modalities and also to assess the long term effectiveness of resin infiltration.

Interpretation of results

The review included two studies, which assessed the effectiveness of arginine and lysine.

The data included in this study comprised of a heterogeneous group of information. Elements that differed across the studies in are: differences in the osteoblasts isolated and cultured.

REPORT ON QUALITY OF EVIDENCE LOOKED UPON:

There are only few articles including in vitro trials, published on the effectiveness of amino acids on osteoblastic activity.resin infiltration in randomized controlled trials. The articles included in this review comprise ofonly in vitro studies. The level of evidence of these articles included in this review is of less quality. However more randomized controlled trials comparing the long term effectiveness of the aminoacids especially in osseointegration needs to be published.

LIMITATIONS:

The present review limits the studies included to be in English language only. This may limit the number of studies assessing the aim of this review. This review also considers only the published data for result interpretation. The unpublished and the raw data of the studies have not been included for interpretation. Due to the heterogeneous nature of the osteoblasts(rats and human normal and osteopenic bone derived osteoblasts) included in this review the pooling of data was not possible.

FUTURE SCOPE:

Studies with increased sample size, standardised method of evaluation and Randomised Controlled Trials with longer follow up periods are needed to compare the

effectiveness ofaminoacids on osteoblasts and bone formation. Therefore, we will be conducting a randomized controlled trial to compare the effectiveness of aminoacids on osteoblasts and bone formation around implants in rabbit model.

SUMMARY

The aim of the systematic review was to evaluate the effectiveness of aminoacids on osteoblastic activity and critically appraise the articles included..

An electronic search was carried out on PUBMED database for the articles which could be used for evaluation.

Article search was narrowed down based up on the pre-stated inclusion and exclusion criteria. A total of 2 articles were included in this systematic review for detailed evaluation.

ALP activity osteocalcin levels nitric oxide levels were considered as the primary outcome variable for assessment in the included study.both articles included in this review showed significant osteoblastic activity after amino acid administration.

Based on the results of this systematic review we can conclude that amino acids definitely have an effect on osteoblastic activity after aminoacid treatment.

CONCLUSION

The osteoblastic proliferation and potential of aminoacids to stimulate osteoblastic activity is well established in this literature review. The evidence regarding the effectiveness of aminoacid is promising but needs further research on bone formation around implants.

BIBLIOGRAPH Y

List Of Included Studies

1. Arginine and L-Lysine stimulation on cultured human osteoblasts. P.Torricelli,M.Fini,G.Giavaresi,S.Gnudi,A.Nicolini,A.Capri.Biomed icine and Pharmacotherapy 56(2002)492-497
2. L-Lysine and L-Arginine on primary osteoblastic cultures from normal and osteopenic rats.M.Fini et al Biomed pharmacotherapy2001;55:213-20
3. Human osteopenic bone-derived osteoblasts: essential amino acidstreatment effects.Torricelli P et al

List Of Excluded Articles

Shivam sinha and Satish Chandra Goel

1.Effect of amino acids lysine and arginine on fracture healing in rabbits:A radiological and histomorphological analysis. Journal of Orthopaedics,2009 Oct-Dec;43(4):328-334.

2. Essential amino acids increase the growth and alkaline phosphatase activity in osteoblasts cultured in vitro.IL Farmaco 56 (2001) 755-761