Alteration of bone metabolism in OLETF rat
Alteration of bone metabolism in OLETF rat

by

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- ABSTRACT -

Alteration of bone metabolism in OLETF rat

**Purpose:** OLETF rat is a model of Type II DM. Bone metabolism has influenced with hormones, growth factors, and cytokines that orchestrate the activities of both osteoclast and osteoblast cells. Type II DM influences bone metabolism and Neuropeptides-leptin and NPY2 receptor’s in hypothalamic control bone formation with osteoblast activity. We tried to distinct alteration of bone metabolism in OLETF rat from 8wks to 52wks.

**Animals and treatment:** Serum samples were collected in eleven male OLETF and LETO (control) rats, femoral BMD and percent body fat were measured in 8, 25, 40, 52wks of age. In 25wks of age, all the OLETF rats developed Type II DM. In 52wks of age, rats were sacrificed and brain sections were made for immunohistochemistry. Hormones, growth factors and bone markers were measured by radioimmunoassay.

**Results:** Body weight and percent body fat in OLETF rat were higher than that of LETO rat in whole time (p<0.001); but BMD was lower than control (p<0.05) from 40wks of age. OLETF rat hypothalamic arcuate neuron cell NPY2 receptor were strongly pattern expressed than LETO rat. OLETF rat testosterone was decreased in 25wks and significantly lower in 40 and 52wks (p<0.05); serum leptin and IGF-I levels were higher (p<0.05) in whole time; insulin levels were higher from 8wks to 40wks(p<0.05), but in 52wks lower (p<0.05) than LETO rat; free T4 were higher in 8wks and 25wks (p<0.05), but in 40wks there was no difference, in 52wks lower than LETO rat; corticosterone level was lower in 8wks (p<0.05), but higher in 25wks, 40wks, and 52wk (p<0.05). OLETF rat serum CTx was higher (p<0.05),
but OPG was lower (p<0.05) in 40wks and 52wks.

**Conclusion:** OLETF rat had low bone density after 40wks of age and this finding could be correlated with low level of testosterone, high level of corticosterone and leptin in serum, and also NPY2-R over-expression in hypothalamic arcuate nuclei.

**Key Words:** Bone metabolism, NPY2 receptor, leptin, hypothalamic arcuate, hormone.
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<table>
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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>DM</td>
<td>diabetes mellitus</td>
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<tr>
<td>BMD</td>
<td>bone mineral density</td>
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<tr>
<td>NPY</td>
<td>neuropeptide Y</td>
</tr>
<tr>
<td>OLETF</td>
<td>Otsuka Long-Evans Tokushima Fatty</td>
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<tr>
<td>LETO</td>
<td>Long-Evans Tokushima</td>
</tr>
<tr>
<td>IPGTT</td>
<td>Intraperitoneal glucose tolerance test</td>
</tr>
<tr>
<td>%CV</td>
<td>percent coefficient of variation</td>
</tr>
<tr>
<td>OPG</td>
<td>osteoprotegerin</td>
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<td>CTx</td>
<td>C-terminal telopeptides</td>
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<tr>
<td>Ob-R</td>
<td>leptin receptor</td>
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<tr>
<td>NPY2-R</td>
<td>neuropeptide Y2 receptor</td>
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<tr>
<td>RANKL</td>
<td>receptor activator of NF-kB ligand</td>
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<tr>
<td>RANK</td>
<td>receptor activator of nuclear factor kappa B</td>
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<tr>
<td>TGF-β</td>
<td>transforming growth factor-β</td>
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I. INTRODUCTION

It has well established that Type I diabetes mellitus (DM) is one of the reason of second osteoporosis (Shires R et al, 1981; Goodman WG and Hori MT, 1984), but Type II DM still has controversial as the reason of the osteoporosis (Weinstock RS et al, 1989; el Miedany YM et al, 1999; Christensen JO and Svendsen OL, 1999; Leidig-Bruckner G and Ziegler R, 2001). Previous study had demonstrated that bone mineral density (BMD) is intimated with body composition, high BMD associated with high body composition (Felson DT et al, 1993).

Otsuka Long-Evans Tokushima Fatty (OLETF) rat is the model of Type II DM, lacking CCK-A receptors are hyperphagic, obese, and diabetic (SHENG BI et al, 2001). OLETF rat’s weight has difference to libitum-fed Long-Evans Tokushima (LETO) rat’s from 5wks old, OLETF rat’s weight is significant higher than LETO rat’s in 40wks old, and the body weight gaps is about 200g (Kawano K et al, 1994; Kawano K et al, 1996). It is well known that level of OLETF rat’s glucose will be elevated from 18wks and will be developed DM in 24wks; albuminuria will be increased from 30wks, and pancreas β-cell secreted insulin’s function will be decreased from 36wks (Kazuya K et al, 1999).

Bone metabolism has influenced with hormones, growth factors, and cytokines that orchestrate the activities of both osteoclast and osteoblast cells. Previous studies showed that neuropeptides and leptin have relationship with bone (Bjurholm A,1991; Herzog H ,2002; Yun-Jung Leea et al, 2002; J Cornish et al, 2002; Melanie Henry et al ,2005). Leptin is a 16-KDa protein, which made by white fat cells, and its concentration in the blood varies with the fat load, which limited by reducing the secretion of hypothalamic eating stimulator,
neuropeptide (James F and Whitfield, PhD 1998). There are four possibilities for leptin controls bone formation: an autocrine, paracrine and/or endocrine mechanism or, as was the case for the rest of function of leptin, a neuroendocrine mechanism. From SHU’s study (SHU TAKEDA and GERARD KARSENTY, 2001), leptin did not act on osteoblasts directly, it acted on osteoblasts through an autocrine, paracrine or endocrine mechanism. Neuropeptide Y (NPY) is a downstream modulator of leptin action, possibly at the level of the arcuate and hypothalamic nucleus where NPY neurons are known to expression both leptin receptors and Y2 receptors. Leptin receptors and NPY2 receptors are present on NPY-expressing neurons of hypothalamic arcuate nucleus and are likely to share some common signaling pathways (Stephens, T.W. et al. 1995; Broberger, C. et al, 1997; Baskin, D.G. et al, 1999; King, P.J. et al, 2000). It had demonstrated that hypothalamic arcuate Leptin and NPY2 receptor control of bone formation with osteoblast activity (Paul A Ballock et al, 2005).

This study was designed to find the distinct alteration of bone metabolism in OLETF rat from 8wks to 52wks.
II. MATERIALS AND METHODS

A. ANIMALS AND TREATMENT

6wks old eleven male OLETF rats and eleven age-matched male LETO rats were obtained as a generous gift of the Tokushima Research Institute, Otsuka Pharmaceutical Tokushima, Japan. Rats were individually housed and maintained on a 12:12-h light-dark cycle (lights on at 8:00 AM) with bottle water available ad libitum, and chows available ad libitum. Body weight was measured monthly, and from the 24wks old, it was measured weekly.

In 8wks, 25wks, 40wks, 52wks after over night fasting, all the rats were deeply anesthetized with U.S.P between AM 9 to AM 12, for were collected serum to measure hormones and bone markers. In 25wks, all the rats were done intraperitoneal glucose tolerance test (IPGTT). After overnight fasting, the rats were anesthetized with U.S.P, blood were collected with 24 gauge catheter in tail vein, and injected 50% glucose in peritoneal 2g/kg (Kazuya K et al, 1999), collected blood after injected glucose 1 hour and 2 hours. The blood was preserved in 4°C about 60minutes, blood samples were centrifuged 15minutes in 4°C with 2500rpm. Serum and plasma were collected and preserved in -70°C deep freezer until biochemical measurement. The plasma glucose levels were measured with blood sugar measuring instrument (Beckman, Stockholm, Sweden). Only rats that satisfied peak plasma glucose >300 mg/dl and plasma glucose at 120min >200mg/dl was diagnosed as Type II DM; rats that satisfied Peak plasma glucose >300 mg/dl or plasma glucose at 120min >200mg/dl was diagnosed impaired glucose tolerance (Kazuya K et al, 1999).
B. PHYSICAL MEASUREMENT

All the rats were deeply anesthetized with ketamine hydrochloride and Zylazine hydrochloride for measure body fat and femoral BMD at the 8wks, 24wks, 40wks and 52wks. It was measured using PIXImus II densitometer (PIXImus™ Series; GE LUNAR, Madison, WI, USA) with 80/35 X-ray source, beam current 500µA, and scan resolution 0.18 x 0.18 mm, specifically designed for small animals. To ensure clinical integrity of the PIXImus, calibration of the instrument was conducted using thirty SD rats were measured twice, Results are given in g/cm² and PIXImus II percent coefficient of variation (%CV) was measured 1.7%. BMD was measured at left femur; total body was sectioned eight part, measure eight sections and combinded.

C. BIOCHEMICAL ANALYSIS

Serum and plasma samples in 8wks, 25wks, 40wks and 52wks were produced radioimmunoassay with rat insulin RIA kit (Linco Research Inc., St Charles, MO, USA), rat leptin RIA kit (Linco Research Inc., St Charles, MO, USA), rat corticosterone RIA kit (DPC Co., Los Angeles, CA, U.S.A), rat IGF-I RIA kit (DSL-2900, DSL.Inc, Texas, USA), human free T4 RIA kit (DPC Co., Los Angeles, CA, U.S.A), human testosterone RIA kit (DPC Co., Los Angeles, CA, U.S.A). Insulin kit intraassay %CV (coefficient of variation) is 2.7%, leptin kit %CV is 1.5%, corticosterone kit %CV is 4.3%, free T4 kit %CV is 5-10%, testosterone kit %CV is 5-18%, IGF-I kit %CV is 3.8-6.1%.
Bone resorption markers osteoprotegerin (OPG) and C-terminal telopeptides (CTx) were produced ELISA assay with Osteoprotegerin for mouse and rat (BI-20602, biomedical Medizinprodukte DMbH, Australia) and RatLapsTM ELIS Y kit (Nordic Bioscience Diagnostics, Denmark). OPG kit intraassay %CV is 5%-10%, CTx kit intraassay %CV is 5.6%.

D. IMMUNOHISTOCHEMISTRY

52wks old eleven LETO rats and eleven OLETF rats were deeply anethetized intra-peritoneally with 5% chloral hydrate solution 100 mg/kg i.p (Fluka 00672. Germany) and perfused via the ascending aorta with 300ml of calcium free Tyrode’s solution (37°C), followed by 300ml (37°C) of mixture of 4% paraformaldehyde and 0.2% picric acid diluted in 0.16 M phosphate buffer (pH 6.9 , Ulrika Smedh et al ,1998; Marie-Louise et al, 1998; PABLO BRUMOVSKY et al, 2005) and 200ml ice cold the same fixative. Brains were removed from the cranium quickly, post fixed for 90minutes at 4°C with 4% paraformaldehyde and 0.16M phosphate buffer, 0.2% picric acid and finally immersed in 10% sucrose diluted in phosphate-buffered saline (PBS; pH 7.4) containing 0.01% sodium azide (Sigma, St. Louis, MO) and 0.02% Bacitracin (Sigma) at 4°C for 24 hours. All tissue was embedded in Tissue-Tek O.C.T compound (Sakura, Torrence, CA) and serially sectioned in a cryostat (Microm, Heidelberg, Germany). Sections were cut 5µm and mounted on saline-coated micro slides, and incubated at room temperature for one hour with goat polyclonal anti-serum to the leptin receptor (ob-R) (diluted 1:100; anti-serum sc-1834; lot, G116; Santa Cruz, CA, USA) and rabbit antibody against the NPY2 receptor (NPY2-R)
After rinsing in phosphate-buffered saline (PBS; 0.1M phosphate buffer, PH 7.4, 0.15M NaCl), sections were incubated for 30 minutes at room temperature respectively with anti-goat immunoglobulins (dilution 1:250; DAKO, Glostrup, Denmark) secondary and LSAB2 system-HRP(DAKO, Carpinteria, CA, USA) secondary antibodies. Immunostaining was visualized by using 3, 3’-diaminobenzidine (DAB) solution (DAKO, CA, USA), and counterstained with Mayer’s hematoxylin.

A semiquantitative assessment for Ob-R and NPY2-R expression was done according to the following criteria: Negative (<5% positive staining of neuron cells), 1+ (5-24% positive staining of neuron cells), 2+ (25-50% positive staining of neuron cells), and 3+ (>76% positive staining of neuron cells).

E. STATISTICAL ANALYSIS

The data were statistically analyzed using SPSS 11.5 software. The continuous variables are expressed as mean ± standard deviation (SD) categorical data were compared using independent t-test. A value of P<0.05 was considered statistically significant.
III. RESULTS

A. BODY WEIGHT AND BODY COMPOSITION

OLETF rat’s weight (Fig. 1.) was significant higher than LETO rat’s weight in the whole time (p<0.001), and the weight gaps were about 200g from 28wks to 40wks. OLETF rat’s body composition (Fig. 2.) was significant lower than LETO rat’s composition in 8wks, but significant higher in 25wks, 40wks and 52wks respectively (37.26±2.17 vs. 42.21±1.26, p<0.001; 44.89±2.26 vs. 38.93±1.44, p<0.001; 44.28±4.28 vs. 39.06±1.64, p<0.001; 44.09±4.85 vs. 42.69±1.62, p<0.001, respectively). OLETF rat’s femur BMD was significant higher than LETO rat’s in 8wks and 25wks (0.198±0.014 vs. 0.161±0.007, p<0.05; 0.285±0.014 vs. 0.267±0.011, p<0.05, respectively), but it was significant lower in 40wks and 52wks (0.284±0.012 vs. 0.293±0.008, p<0.05; 0.258±0.010 vs. 0.285±0.011, p<0.05, respectively) (Fig. 3.).

B. FASTING GLUCOSE AND HORMONES

In 25wks, 40wks and 52wks, OLETF rat’s fasting glucose was significantly higher than LETO rat’s (p<0.05). There was no difference between LETO rat’s insulin and OLETF rat’s insulin in 8wks; OLETF rat’s insulin was significant higher than LETO rat’s in 25wks and 40wks (p<0.05); OLETF rat’s serum insulin was significant lower than LETO rat’s in 52wks (p<0.05). OLETF rat’s insulin was increased in 25wks and decreased in 40wks (p<0.05); LETO rat’s insulin was no significant difference during study. OLETF rat’s leptin was significant higher than LETO rat’s leptin in whole time (Table. 1), and the peak of OLETF rat’s leptin was found in 25wks.
OLETF rat’s serum testosterone was decreased in 25wk and definite lower in 40wks and in 52wks (p<0.05); OLETF rat’s serum IGF-I levels was significant higher than LETO rat’s in the whole time (p<0.05); OLETF rat’s free T4 was significant higher than LETO rat’s in 8wks and 25wks (p<0.05); there was no significant difference in 40wks between two groups; OLETF rat’s free T4 was significant lower than LETO rat’s in 52wks (p<0.05); OLETF rat’s corticosterone was significant lower than LETO rat’s in 8wks (p<0.05), but significant higher in 25wks, 40wks and 52wks (p<0.05)(Table.1).

C. Ob-R AND NPY2-R PROTEIN EXPRESSION IN HYPOTHALAMIC ARCUATE

In hypothalamic arcuate of rat brain tissues Ob-R and NPY2-R consistently expressed in the membrane and cytoplasm of the neuron cells. Ob-R protein more highly expressed in LETO rat’s hypothalamic arcuate neuron cells but not statistically significant (p=1.000). LETO rat’s exhibited a variable degree for Ob-R protein expression: 3(+) in 27.2%, 2(+) in 18.1% and 1(+) in 54.5%. In OLETF rat’s brain tissues 3(+) Ob-R was seen only in 18.1% of cases, 2(+) and 1(+) was seen in 36.3% and 45.5% of cases respectively. NPY2-R protein significantly higher in OLETF rat’s hypothalamic arcuate neuron cells than LETO rat’s (p=0.017). In OLETF rat’s, 3(+) expression of NPY2 was seen in 54.5% OLETF rat’s hypothalamic arcuate neuron cells, but in LETO rat’s, there was no case showed 3(+) immunoreactivity of expression of NPY2-R. (Fig. 4, Table. 2)

D. BONE MARKERS

OLETF rat’s and LETO rat’s serum CTx were no significant difference in 8wks and
25wks (38.87±5.50 vs. 34.06±5.14, p>0.05; 29.30±5.67 vs. 29.26±7.11, p>0.05, respectively); but OLETF rat’s serum CTx was significant higher than LETO rat’s serum CTx from 40wks (29.90±9.20 vs. 19.20±3.81, p<0.05; 32.71±5.35 vs. 26.24±1.83, p<0.05, respectively) (Fig. 5). OLETF rat’s and LETO rat’s serum OPG were no significant difference in 8wks and 25wks; OLETF rat’s was significant lower than LETO rat’s in 40wks and 52wks (35.39±15.03 vs. 46.38±19.18, p<0.05; 72.39±20.56 vs. 70.55±43.935, p<0.05) (Fig. 6).
IV. DISCUSSION

Bone metabolism, which was influenced by hormones, growth factors, and cytokines, orchestrate the activities of both osteoclast and osteoblast cells. Bone mass is maintained through a balance between bone formation and resorption, there are three possible mechanisms by which a lower bone volume can be achieved, the first is to decrease bone formation, the second is to increase bone resorption, and the third is combination of the first and the second. OLETF rat’s body weight and body composition was significant higher than LETO rat’s body weight and body composition, but OLETF rat’s bone mineral density was significant lower LETO rat’s from 40wks in this study.

Sex steroids and parathyroid hormone are well known hormones in regulating bone resorption. No hormone has been shown to control bone formation. As type II DM model, male OLETF rat was developed DM in 24wks, and the function of insulin secretion was reduced from 36week. In patients with Type II DM, male hypogonadism has been reported (Fushimi H et al, 1989). Although obesity or Type II DM is closely related to gonadal dysfunctions, details of the mechanism remain unclear (Kawano K et al, 1992). It is also well known, when the serum testosterone decreased, the osteoblast cell production of transforming growth factor-β (TGF-β) was also decreased. TGF-β treatment increase bone formation parameters in vitro and in vivo and decreases osteoclaste cell survival in vitro. In this study, all of the OLETF rats are developed DM in 25wks, serum testosterone also decreased in 25wks. In 40wks and 52wks, that was the similar as the former study (KATSUMORI KOMAKI et al, 2005), and it leads to increase of bone resorption. Because type II DM didn’t influence on parathyroid hormones, so we didn’t measure the parathyroid
hormone in this study.

Conflicting reports existed in regarding the influence of impaired insulin secretion and metabolic control on bone metabolism (McNair P et al, 1979; Munoz-Torres M et al, 1996). In vivo, local insulin treatment to the hemicalvaria in nondiabetic mice increased osteoid volume and the number of osteoblasts (Cornish J et al, 1996). High extracellular glucose levels have been found to cause changes in osteoblastic gene expression (Hough S et al, 1981), inhibit osteoblast-like cell growth (Terada M et al, 1998) and promote osteoclastic bone resorption (Williams JP et al, 1997). From the change of OLETF rat’s insulin and fasting glucose in this study, we can infer that decreased insulin may decrease bone formation, high level of glucose may increase bone resorption.

Serum IGF-I was correlated with body composition and BMD has been confirmed in former study (H. N. Rosen et al, 1995). The IGF-I circulated in blood and interaction on epiphyseal growth plate increased unilateral bone growth significantly (J. Isgaard et al, 1986). In this study, OLETF rat serum IGF-I was higher than LETO in the whole time before 40wks, but decreased in 40wks indicated that bone formation may also decreased from 40wks.

It is well known that glucocorticoid hormones increase bone resorption. Corticosterone is the main glucocorticoid hormone in rat, and the high level of corticosterone decrease bone formation; in human, glucocorticoid decrease osteoblast differentiation and increase bone loss. In this study, OLETF rat’s corticosterone was significant higher than LETO rat’s from 40wks to 52wks, so corticosterone may also influence the BMD.

Exceeded thyroid hormone stimulates bone resorption, resulting in increased bone turnover and both cortical and trabecular bone loss (Meunier PJ et al, 1972; Mosekilde L and Melsen F, 1978). In this study, the free T4 have no significant difference between two groups.
in 40wks, indicated that free T4 may have no influence in bone metabolism of OLETF rat.

Leptin is almost exclusively produced by fat with a very strong association between serum leptin and fat mass (Maffei M et al, 1995; Considine RV et al, 1996; Thomas T et al, 2000). Leptin’s effect on BMD is complex and likely to be mediated through direct and indirect mechanisms (Reseland JE and Gordeladze JO, 2002; Ruhl C and Everhart J, 2002; Reid IR and Comish J, 2004). Previous study showed that as a potent inhibitor of bone formation, leptin acting through the central nervous system in mice (Ducy et al, 2000), it enhances osteoblast formation and inhibits osteoclast generation (Holloway WR et al, 2002; Cornish J et al, 2002). Centrally, leptin has been shown to inhibit bone formation through a hypothalamic relay, an effect that is inhibited using blockers (Ducy P et al, 2000; Cock TA and Auwerx J, 2003). The leptin pathway acts both to stimulate bone formation when serum leptin levels are absent and to suppress bone formation when present in excess (Ducy, P. et al, 2000). OLETF rat serum leptin levels was significant higher than LETO rat from 8wks to 52wks, and in 8wks and in 25wks was significant higher BMD than LETO rat indicated that 8wks and 25wks serum leptin wasn’t exceeded, but from 40wks was exceeded. NPY2-R expressed strongly pattern in OLETF rat hypothalamic arcuate NPY2 was mediated with serum leptin, when leptin was higher, NPY2-R was over expressed, and it decreased bone formation. From Paul’s study (Paul A. Baldock et al, 2002), hypothalamic Y2 receptor deletion were significantly increased bone formation in mice. In this study, high level of serum leptin was observed from 8wks to 25wks indicated that serum leptin in this period was very important in evaluation of BMD. NPY2 in hypothalamic arcuate functions is also important in evaluation of BMD in post 40wks.

OPG, as one of the tumor necrosis factor receptor, which with it’s ligand –receptor
activator of NF-kB ligand (RANKL) regulation osteoclast specialization (Yasuda H et al, 1998). RANKL was position on membrain and combination with osteoclast progenitor receptor activator of nuclear factor kappa B (RANK), increase the osteoclast cell’s formation and activity. RANKL’s bone resorption activity was regulated with OPG, and OPG was combination with RANKL, and interrupt RANKL and RANK interaction than control inhibit osteoclast (Suda T et al, 1999; Hofbaner LC et al, 2000). From OPG level lower in OLETF rat than LETO rat in 40wks and 52wks, we found that the bone resorption in OLETF rat was more activity than LETO rat. CTx was formed depend on cathepsin K which is important enzyme of bone’s collegen resolution (Bonde M et al, 1997), because the high level of CTx was reflection excellent of resorption. OLETF rat’s CTx was higher than LETO in 40wks and 52wks in this study, both of bone resorption marker reflection that OLETF rat resorption was excellent in 40wks and 52wks than the LETO rat, it also indicated that OLETF rat’s bone resorption more activity than LETO rat from 40wks.

For the limitation of this study, body composition was measured with PIXImusII which is the model of small animal, the rat body was sectioned eight parts, and the data were combinded. All the rats were anethetized more than ten times and blood sample were collected five times, rat’s body weight was not increased significantly like other studies.
VI. CONCLUSION

OLETF rat had low bone density after 40wks of age and this finding could be correlated with low level of testosterone, high level of corticosterone and leptin in serum, and also NPY2-R over-expression in hypothalamic arcuate nuclei.
Fig. 1. OLETF rat’s body weight higher than LETO in the whole time (P<0.001).

Fig. 2. OLETF rat’s body composition was higher in 25wks, 40wks and 52wks (P<0.001).

Fig. 3. OLETF rat’s femur BMD was lower than LETO (P<0.05) in 40wks and 52wks.
Table 1. Fasting glucose, Insulin, Leptin, Testosterone, IGF-I, free T4, and Corticosterone in 8wks, 25wks, 40wks and 52wks.

<table>
<thead>
<tr>
<th></th>
<th>LETO (n=11)</th>
<th>OLETF (n=11)</th>
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<td>Glucose (mg/dl)</td>
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<tr>
<td></td>
<td>120.46±6.64</td>
<td>135.78±7.74*</td>
<td>159.86±8.54</td>
<td>232.97±18.77*</td>
<td>131.27±23.50</td>
<td>322.26±133.96*</td>
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<tr>
<td>Insulin (ng/ml)</td>
<td>0.549±0.154</td>
<td>0.585±0.100</td>
<td>0.73±0.29</td>
<td>1.931±0.493*</td>
<td>0.60±0.187</td>
<td>0.761±0.43#</td>
<td>0.780±0.209</td>
<td>0.631±0.146</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>3.12±1.04</td>
<td>7.04±1.52#</td>
<td>10.68±2.05*</td>
<td>29.79±7.77**</td>
<td>4.31±0.79*</td>
<td>11.93±5.21**</td>
<td>6.89±0.89#</td>
<td>5.89±2.63#</td>
</tr>
<tr>
<td>Testosterone (ng/dL)</td>
<td>321.4±151.2</td>
<td>349.7±115.9</td>
<td>226.0±94.1</td>
<td>176.5±32.9</td>
<td>218.7±74.5</td>
<td>101.6±36.5**</td>
<td>166.7±67.6</td>
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</tr>
<tr>
<td>IGF-I (ng/ml)</td>
<td>1011.6±44.3</td>
<td>1288.4±91.2*</td>
<td>1043.5±69.4</td>
<td>1373.7±155.7</td>
<td>825.6±34.0*</td>
<td>1297.4±158.4*</td>
<td>1555.4±141.9*</td>
<td>1825.6±155.9**</td>
</tr>
<tr>
<td>free T4 (ng/dL)</td>
<td>1.284±0.089</td>
<td>1.548±0.165*</td>
<td>1.051±0.113*</td>
<td>1.434±0.087**</td>
<td>1.048±0.071</td>
<td>1.053±0.079*</td>
<td>0.608±0.073*</td>
<td>0.423±0.082**</td>
</tr>
<tr>
<td>Corticosterone (ng/ml)</td>
<td>405.6±41.4</td>
<td>262.2±51.7*</td>
<td>195.3±25.8*</td>
<td>241.4±55.1*</td>
<td>350.0±61.2*</td>
<td>415.9±81.4*</td>
<td>201.3±34.9*</td>
<td>351.2±63.3*</td>
</tr>
</tbody>
</table>

* : p < 0.05 compared to LETO.  
# : p < 0.05 compared to base line
Fig. 4. Ob-R and NPY2-R immunostaining in LETO and OLETF brain tissues.

(LSAB, ×200)

A, D: Increased cytoplasmic and membrane expression for Ob-R in LETO.

B, C: Weekly positive cytoplasmic and membrane staining for Ob-R in OLETF.

Table 2. Expression of Ob-R in LETO (A) and OLETF (B) (P=1.000), NPY2-R in LETO (C) and OLETF (D) (P=0.017).

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Ob-R LETO</td>
<td>6 (54.5%)</td>
<td>2 (18.1%)</td>
<td>3 (27.2%)</td>
</tr>
<tr>
<td>Ob-R OLETF</td>
<td>5 (45.5%)</td>
<td>4 (36.3%)</td>
<td>2 (18.1%)</td>
</tr>
<tr>
<td>NPY2-R LETO</td>
<td>6 (54.5%)</td>
<td>5 (45.6%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>NPY2-R OLETF</td>
<td>3 (27.3%)</td>
<td>2 (18.1%)</td>
<td>6 (54.5%)</td>
</tr>
</tbody>
</table>
Fig. 5. OLETF rat’s CTx was higher than control in 40wks.

* compare to LETO in the same week (P<0.05), # compare to base line (P<0.05).

Fig. 6. OLETF rat’s OPG was lower than control in 40wks (P<0.05).

* compare to LETO in the same week (P<0.05), # compare to base line (P<0.05).
REFERENCES


23. James F. Whitfield, PhD, Leptin—A New Member of the Bone Builders’ Club? FRSC.

24. J. Cornish, K E Callon, U Bava et al. Leptin directly regulates bone cell function in *vitro* and reduces bone fragility in *vivo* *Journal of Endocrinology* 175:405–415, 2002


30. KATSUMORI KOMAKI, YASUHIRO OHNO and NORIHIKO AOKI. Gonadal hormones and gonadal function in type 2 diabetes model OLETF(Otsuka Long Evans Tokushiwa Fatty)Rats. Endocrine Journal 52(3):345-351, 2005


41. PABLO BRUMOVSKY, DAVOR STANIC, SAM SHUSTER et al. Neuropeptide Y2 Receptor Protein Is Present in Peptidergic and Nonpeptidergic Primary Sensory Neurons of the Mouse. *THE JOURNAL OF COMPARATIVE NEUROLOGY* 489:328–348, 2005


43. Reid IR, Comish J. Direct actions of leptin on bone remodeling. *Calcif Tissue Int* 74:313–316, 2004


52. Suda T, Takahashi N, Udagawa N et al. Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. *Endocrine Rev* 20: 345-357, 1999


OLETF쥐 골대사 변화

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배경: OLETF쥐는 제2형 당뇨병 모델 쥐이다. 골대사는 호르몬이나 성장인자, cytokines, 조글로세포 공급체포의 활성과 영향을 받는다. 제2형 당뇨병은 골대사에 영향을 주며 hypothalamic arcuate의 렼빈과 NPY2은 조글로세포의 활성을 조절함으로써 빠른 생성을 지배 한다. 본 연구의 목적은 OLETF쥐와 LETO쥐에서 8주에서 52주 사이의 골대사 변화를 관찰하는 것이다.

대상 및 방법: 11마리의 OLETF 오성 쥐와 연령을 맞춘 11마리의 LETO 오성 쥐를 대상으로 하였다. 8주, 25주, 40주, 52주에 마취한 후 체험하하여 혈청을 얻고 골밀도와 제조성을 측정하였다. 25주에는 IPGTT를 시행하여 모든 OLETF쥐에서 당뇨병이 진행되었음을 확인하였다. 52주에 쥐를 채워서 케터의 hypothalamic arcuate에서 렼빈과 NPY2 수용체의 발현을 면역조직학방법으로 관찰하였다. 혈청에서 각종 호르몬과 성장인자, 골대사 표지자 등을 측정하였다.

결과: 체중, 체조성은 모든 시기에 OLETF쥐에서 LETO쥐보다 높았다 (P<0.001). 그러나 골밀도는 40주부터 대조군보다 통계적으로 의미있게 높았다 (p<0.05). OLETF귀 뇌의 hypothalamic arcuate의 NPY2의 수용체는 LETO귀보다 강하게 발현되었다. OLETF귀의 테스토스테론은 25주부터 감소하다가 40주와 52주에는 통계적으로 명확하게 LETO귀보다 낮아졌다 (P<0.05). OLETF쥐의 혈청내의 렼빈, IGF-I와 인슐린의 농도는 모든 시기에 대조군보다 높았다 (P<0.05), free T4는 8주와 25주에는 높았다가 (P<0.05), 40주에는 대조군과 차이를 보이지 않았으며, 52주에는 오히려 높았다. 코르티코스테론은 8주에는 낮으나 (P<0.05), 25주, 40주, 52주에는 대조군보다 높았다 (P<0.05). 40주와 52주의 OLETF쥐의 혈청 CTx 는 대조군보다 높으나 (P<0.05), OPG는 오히려 높았다 (P<0.05).
결론: OLETF쥐에서 40주부터 골밀도가 낮게 나타난 것은 혈액내의 고농도의 램틴, 그리고 hypothalamic arcuate에서 NPY2수용체의 과발현과 연관이 있으며, 40주부터 성선기능이 저하되고 코르티코스테로이드분비와 혈액내의 램틴과 hypothalamic arcuate의 NPY2로 인한 골흡수 증가를 감당할 수 없기 때문으로 생각된다.

핵심되는 단: 골대사, NPY2 수용체, 램틴, arcuate, hypothalamic, 호르몬
Alteration of bone metabolism in OLETF rat
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by

Shen Yingji

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in Partial Fulfillment of the Requirements for the Degree of

MASTER OF MEDICAL SCIENCES

Supervised by
Yoon-Sok Chung, M.D., Ph.D.

Department of Medical Sciences
The Graduated School, Ajou University
August, 2006
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