LGD-4033 builds muscle and bone with reduced prostate activity and may be beneficial in age-related frailty

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INTRODUCTION

Steroidal androgens have beneficial effects on muscle and bone but are not widely used due to potential safety concerns. We have discovered a non-steroidal, orally available selective androgen receptor modulator (SARM), LGD-4033. We have characterized the *in vitro* binding and transcriptional activity of LGD-4033 and its effects on bone, muscle, and off target tissues *in vivo*. We have examined the pharmacokinetic tissue distribution to further understand the tissue selective activity of this molecule.

MATERIALS AND METHODS

Receptor Binding and Transcriptional Activity
LGD-4033 was synthesized at Ligand Pharmaceuticals. Competitive binding assays were performed using recombinant baculovirus-expressed AR and radiolabeled DHT to determine an inhibition constant (Ki) for LGD-4033. Transcriptional activity was measured in CV1 cells transiently transfected with human AR expression plasmid and luciferase reporter plasmid containing a AR responsive promoter. Similar experiments were carried out with related steroid receptors. These methods have been previously described in more detail.[1,2]

Animal Studies
Mature male and female Sprague-Dawley rats from Harlan were used following a 1 week acclimation period. The experimental protocol was approved by the Institutional Animal Care and Use Committee, and the animals were maintained in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals.

Orchidectomized Male Rat Study Design
Male rats were castrated (ORDX) and sorted into groups (n=4) based upon body weight. Rats began treatment 14 days after surgery and treatment continued for 14 consecutive days. Rats received either vehicle or LGD-4033 by once daily oral gavage. The levator ani muscle and the ventral prostate were weighed at necropsy to assess the tissue-selective activity of the compounds. In a second experiment LGD-4033 was orally dosed beginning immediately after castration in male rats (n=3/group). Rats were dosed for 14 days and sacrificed at 2, 4, or 8 hours post dosing on the final day to determine tissue concentration of LGD-4033. LGD-4033 concentrations in plasma and tissue were measured by liquid chromatography with tandem mass spectrometry detection (LC-MS/MS) after acetonitrile extraction.

Intact Female Rat Study Design
Female rats (n=10) were treated with LGD-4033 (3 mg/kg) or vehicle for 28 days. Muscle weights were collected at necropsy. Mean muscle fiber cross-sectional area was measured from paraffin-embedded, H&E stained, histological sections of the mid-gastrocnemius.

Ovariectomized Female Rat Study Design
Female rats were ovariectomized (OVX) and left to develop osteopenia for 8 weeks. Rats were sorted into groups (n=9) based upon dual energy x-ray absorptiometry (DEXA) scans of the femur. Rats received once daily treatment with vehicle or LGD-4033 by oral gavage. Treatment continued for 12 weeks at which point tissue weights were collected at necropsy. DEXA scans, bone histomorphometry, and biomechanical testing were performed on the femur and lumbar spine.

RESULTS

Table 1. *In vitro* binding and transcriptional activity of LGD-4033

Receptor	Receptor Binding K_i (nM)	Transcriptional Activity Efficacy (%)	Transcriptional Activity EC_{50} (nM)
Androgen Receptor	0.9	132	4.4
Mineralocorticoid Receptor	>10,000	1	--
Glucocorticoid Receptor	5,797	1	--
Estrogen Receptor α	nd	1	--
Progesterone Receptor	4,009	1	--

Figure 1. LGD-4033 is a tissue selective androgen in chronically hypogonadal male rats. Male rats were castrated and left untreated for 14 days to develop muscle and prostate atrophy. ORDX rats were treated with LGD-4033 (p.o.) or vehicle for 14 days. LGD-4033 had potent muscle activity but weaker partial agonist activity on the prostate. Data are expressed relative to intact (100%) and ORDX controls (0%).

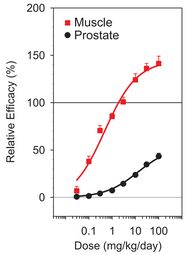


Figure 2. Tissue selectivity is independent of local tissue concentration in acutely hypogonadal male rats. LGD-4033 (30 mg/kg) was administered to ORDX male rats for 14 days beginning immediately after surgery. Tissue concentration of LGD-4033 was measured at necropsy and was greater in prostate compared to muscle at all measured time points. This is in spite of reduced activity on the ventral prostate compared to sham operated intact animals.*p<0.05 vs. sham, †p<0.05 vs. vehicle.

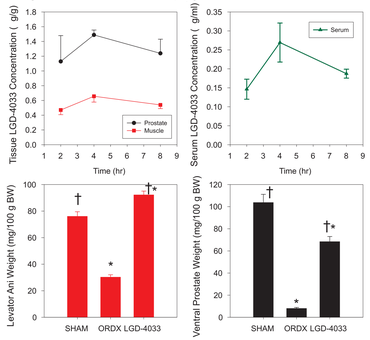


Figure 3. LGD-4033 increases skeletal muscle mass at multiple sites in female rats. LGD-4033 (3 mg/kg) was administered for 28 days to female rats. Wet muscle weight was recorded at necropsy. *p<0.05 vs. vehicle control

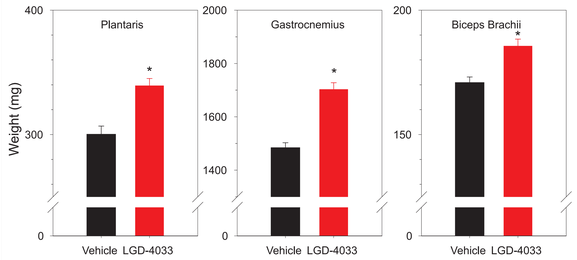


Figure 4. LGD-4033 increased muscle mass is related to increased muscle fiber cross sectional diameter in female rats. A trend towards larger fiber diameter was observed after 28 days.

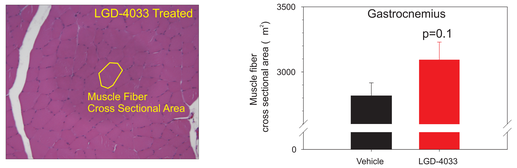


Figure 5. LGD-4033 has beneficial effects on cortical bone in OVX female rats. LGD-4033 increased whole femur BMD as effectively as estradiol and testosterone, but only the androgens increased periosteal bone formation and femur bending strength. *p<0.05 vs. sham, †p<0.05 vs. vehicle.

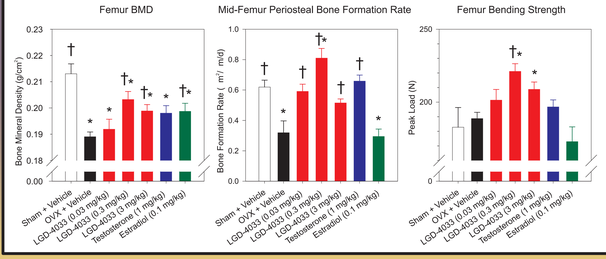
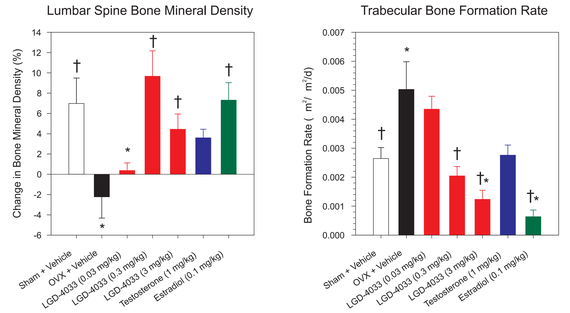


Figure 6. LGD-4033 has beneficial effects on cancellous bone in OVX female rats. LGD-4033 increased lumbar spine BMD as effectively as estradiol and testosterone. LGD-4033 significantly decreased trabecular bone turnover. *p<0.05 vs. sham, †p<0.05 vs. vehicle.



REFERENCES

1. Miner *et al*. **Endocrinology**, 2007. 148(1):363-373.
2. McDonnell *et al*. **J Biol Chem**, 1994. 269(16):11945-9.

SUMMARY

- LGD-4033 is a selective androgen receptor modulator.
- LGD-4033 has anabolic activity in muscle and bone with partial agonist activity on the prostate.
- The tissue selective properties of LGD-4033 are independent of local drug concentration indicating that tissue selectivity is inherent to the compound.
- Studies in animal models suggest that LGD-4033 will be of therapeutic benefit in the frail elderly as well as a variety of other conditions involving sarcopenia, *e.g.*, cancer cachexia, COPD, and chronic kidney disease.
- Based upon its favorable pre-clinical profile, LGD-4033 initiated Phase I clinical trials in 2009.