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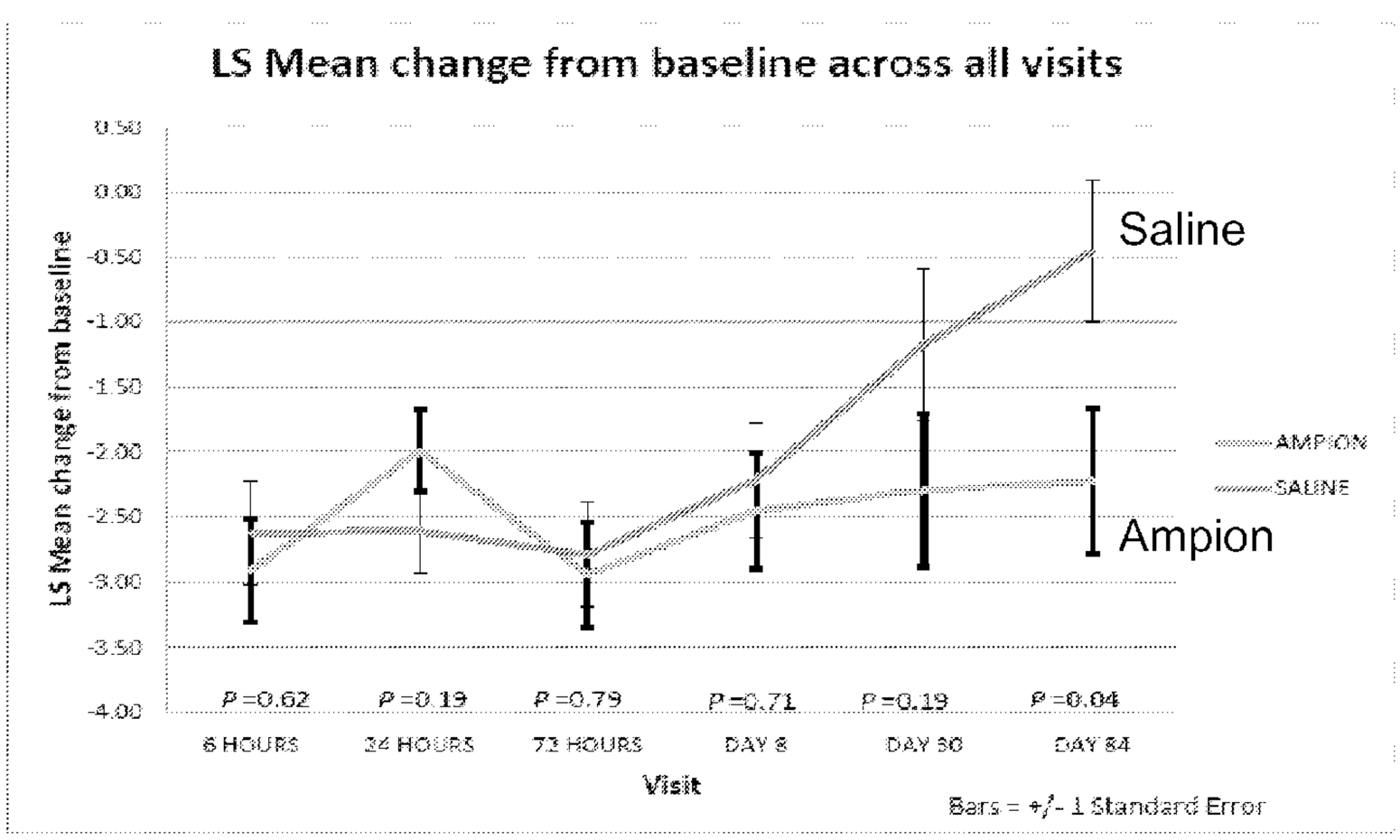
(54) **Titre : TRAITEMENT DE MALADIE DEGENERATIVE ARTICULAIRE**
(54) **Title: TREATMENT OF DEGENERATIVE JOINT DISEASE**

Figure 7



(57) **Abrégé/Abstract:**
The invention provides a method of treating a degenerative joint disease. The method comprises administering an effective amount of a pharmaceutical composition comprising a diketopiperazine with amino acid side chains of aspartic acid and alanine (DA-DKP). The invention also provides a pharmaceutical product as well as a kit comprising DA-DKP.

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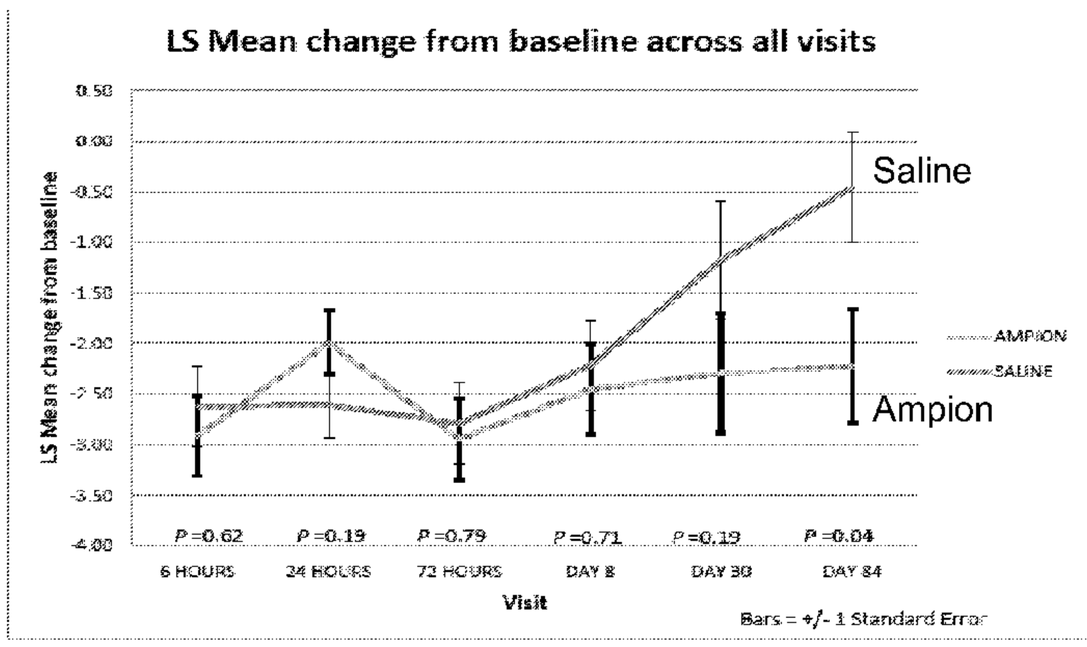
Figure 7



(57) Abstract: The invention provides a method of treating a degenerative joint disease. The method comprises administering an effective amount of a pharmaceutical composition comprising a diketopiperazine with amino acid side chains of aspartic acid and alanine (DA-DKP). The invention also provides a pharmaceutical product as well as a kit comprising DA-DKP.

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TREATMENT OF DEGENERATIVE JOINT DISEASE

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority under 35 U.S.C. §119(e) to U.S. Provisional Patent Application No. 61/545,474, filed October 10, 2011 and U.S. Provisional Patent Application No. 61/561,221, filed November 17, 2011. The entire disclosures of each U.S. Provisional Patent Applications Nos. 61/545,474 and 61/561,221 are incorporated herein by reference.

FIELD OF INVENTION

The invention relates to a method of treating a degenerative joint disease. The method comprises administering an effective amount of a pharmaceutical composition comprising a diketopiperazine with amino acid side chains of aspartic acid and alanine (DA-DKP). The invention also provides a pharmaceutical product comprising DA-DKP.

BACKGROUND

Osteoarthritis is the most common form of arthritis, affecting 25 to 35 million people in the U.S. Chronic pain and disability of osteoarthritis is initially caused by inflammatory responses in joint cartilage and bone that gradually worsens over time. Symptomatic osteoarthritis of the knee occurs in 10 to 13% of persons aged 60 and over. Knee osteoarthritis alone increases the risk of loss of mobility, such as needing assistance walking or climbing stairs, greater than for any other medical condition in people aged 65 and over.

Current drug treatment for osteoarthritis of the knee is limited to analgesics, non-steroidal anti- inflammatory drugs (NSAIDs) and intra-articular steroid injections, all of which have significant limitations due to adverse effects. Despite these medical treatments, chronic knee osteoarthritis often causes progressive disability requiring total joint replacement. The increasing prevalence of osteoarthritis of the knee due to aging and obese populations suggests a growing clinical need for safe and effective local knee treatments that will delay and potentially eliminate the need for more extensive surgical treatments.

SUMMARY OF INVENTION

One embodiment of the invention relates to a method of treating a degenerative joint disease by administering to an animal in need thereof an effective amount of a pharmaceutical composition comprising DA-DKP. In one aspect, the degenerative joint

disease is osteoarthritis.

In another aspect, the composition is administered no more frequently than once every six months, once every 5 months, once every 4 months, once every 3 months, or once every 2 months.

In yet another aspect, the composition can be administered by various administration routes. For example, the administration route may be by local administration, topical administration, or injection. In one aspect, administration by injection is by intra-articular injection. In yet another aspect, the composition administered by intra-articular injection is a composition having a concentration of DA-DKP from about 50 μM to about 350 μM.

In still another aspect, the composition further includes N-acetyl-tryptophan (NAT), caprylic acid, caprylate or combinations thereof. In yet another aspect, the concentration of NAT, caprylic acid, caprylate or combinations thereof in the composition may be about 4 mM to about 20 mM.

In yet other aspects of the method, the DA-DKP is in a composition prepared by removing albumin from a solution of a human serum albumin composition. For example, the step of removing can be by treating the human serum albumin composition by a separation method. Such separation methods can include ultrafiltration, sucrose gradient centrifugation, chromatography, salt precipitation, and sonication. In addition, the step of removing can be by passing the human serum albumin composition over an ultrafiltration membrane with a molecular weight cut off that retains the albumin, and the resulting filtrate contains the DA-DKP. In one aspect, the ultrafiltration membrane has a molecular weight cutoff of less than 50 kDa. In still another aspect, the ultrafiltration membrane has a molecular weight cut off less than 40 kDa, less than 30 kDa, less than 20 kDa, less than 10 kDa, less than 5 kDa or less than 3 kDa. In still another aspect, this composition further comprises NAT, caprylic acid, caprylate or combinations thereof. In yet another aspect, the concentration of NAT, caprylic acid, caprylate or combinations thereof in the composition may be about 4 mM to about 20 mM.

In another aspect, the method of the invention can further include administering a second drug. For example, the second drug can be an analgesic, an anti-inflammatory drug, or combinations thereof.

Another embodiment of the invention is a pharmaceutical product comprising a DA-DKP-containing composition formulated for administration by injection. In one aspect, the product is formulated for administration by intra-articular injection. In another aspect the DA-DKP is prepared by removing albumin from a solution of a human serum albumin composition. In one aspect, the step of removing the albumin can be by treating the human serum albumin composition by a separation method. For example, the separation method can be ultrafiltration, sucrose gradient centrifugation, chromatography, salt precipitation, or sonication. In addition, the step of removing can be by passing the human serum albumin composition over an ultrafiltration membrane with a molecular weight cut off that retains the albumin, and the resulting filtrate contains DA-DKP. In one aspect, the ultrafiltration membrane has a molecular weight cutoff of less than 50 kDa. In still another aspect, the ultrafiltration membrane has a molecular weight cut off less than 40 kDa, less than 30 kDa, less than 20 kDa, less than 10 kDa, less than 5 kDa or less than 3 kDa.

In yet another aspect, the DA-DKP of the pharmaceutical product that is administered by intra-articular injection is a composition having a concentration of DA-DKP from about 50 μM to about 350 μM.

In still another aspect, the product contains the DA-DKP composition that further contains NAT, caprylic acid, caprylate or combinations thereof. In yet another aspect, the concentration of NAT, caprylic acid, caprylate or combinations thereof in the product may be about 4 mM to about 20 mM.

In yet another aspect, the pharmaceutical product, further contains a pharmaceutically-acceptable carrier.

Another embodiment of the invention relates to a kit that includes a pharmaceutical product. In one aspect the product includes a DA-DKP-containing composition formulated for administration by injection. In still another aspect, the DA-DKP in the kit is prepared by removing albumin from a solution of a human serum albumin composition.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the mean change in pain numerical rating scale (NRS) for subjects treated with a single 10 ml injection in one knee of Ampion™ (<5000MW fraction) combined with a steroid (betamethasone)/lidocaine suspension or injected with a saline placebo combined with steroid (betamethasone)/ lidocaine suspension. The scores were

completed at 6 hours post-dose on Day 1, 24 hours post-dose on Day 2 and 72 hours post-dose on Day 4.

Fig. 2 shows the mean change in pain NRS as described in Figure 1 and includes a repeated measures analysis of variance (ANOVA).

Fig. 3A shows the absolute difference in WOMAC (Western Ontario McMaster University Osteoarthritis Index 3.1) pain subscores at 24 hours for the treatment described in Figure 1.

Fig. 3B shows the absolute difference in WOMAC pain subscores at 72 hours for the treatment described in Figure 1.

Fig. 4A shows the absolute difference in WOMAC stiffness subscores at 24 hours for the treatment described in Figure 1.

Fig. 4B shows the absolute difference in WOMAC stiffness subscores at 72 hours for the treatment described in Figure 1.

Fig. 5A shows the absolute difference in WOMAC function subscores at 24 hours for the treatment described in Figure 1.

Fig. 5B shows the absolute difference in WOMAC function subscores at 72 hours for the treatment described in Figure 1.

Fig. 6 shows a potential steroid time course.

Fig. 7 shows the mean change from baseline across all subject visits for the treatment described in Figure 1. ("LS" stands for least squares)

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a method of treating a degenerative joint disease. The treatment comprises administering an effective amount of a pharmaceutical composition comprising aspartyl-alanyl diketopiperazine (DA-DKP) to an individual having a need thereof. DA-DKP has multiple anti-inflammatory and immune modulating effects including inhibition of multiple pro-inflammatory cytokines, chemokines and signaling molecules at the transcription level, inhibition of the migration and adhesion of T-cells and monocytes, activity at the G-coupled protein receptor level, activity on actin-dependent cytoskeletal events, reduction in vascular permeability and inhibition of inflammation induced by platelet activating factor. As described in more detail below, the effects of DA-DKP on degenerative joint disease have been found to be unexpectedly long lasting and in some studies were found to increase in time as compared to the use of

steroids.

The invention also provides for a pharmaceutical product comprising a DA-DKP composition. The DA-DKP of the product may be prepared by removing albumin from a solution of human serum albumin.

The invention also provides for kit comprising a DA-DKP composition formulated for administration by injection.

A degenerative joint disease is a gradual deterioration of the articular cartilage that covers joints. A degenerative joint disease (osteoarthritis) is a noninfectious progressive disorder of the weightbearing joints. The normal articular joint cartilage is smooth, white, and translucent. It is composed of cartilage cells (chondrocytes) imbedded in a sponge-like matrix made of collagen, protein polysaccharides, and water. With early primary arthritis, the cartilage becomes yellow and opaque with localized areas of softening and roughening of the surfaces. As degeneration progresses, the soft areas become cracked and worn, exposing bone under the cartilage. The bone then begins to remodel and increase in density while any remaining cartilage begins to fray. Eventually, osteophytes (spurs of new bone) covered by cartilage form at the edge of the joint. As mechanical wear increases, the cartilage needs repairing. The cartilage cells are unable to produce enough of the sponge-like matrix and therefore the damaged cartilage cannot repair itself. The cartilage has no blood supply to enhance healing. The majority of degenerative joint disease is the result of mechanical instabilities or aging changes within the joint. This includes old age degenerative arthritis and, in younger individuals, may be the result of injuries, bruises, abnormal joint configuration (i.e. hip dysplasia), or mechanical wear from anterior cruciate ligament rupture, patellar luxation, or osteochondritis dissecans, for example. Degenerative joint disease can occur at any joint in the body, including without limitation, knee, hip, shoulder, hand and spine.

Conventional pharmaceutical therapies for degenerative joint disease include acetaminophen, nonsteroidal anti-inflammatory drugs (NSAIDS), narcotics, and corticosteroids.

"Treat" is used herein to mean to reduce (wholly or partially) the symptoms, duration or severity of a disease.

The pharmaceutical composition comprising DA-DKP of the invention is administered to an animal in need of treatment. Preferably, the animal is a mammal, such

as a rabbit, goat, dog, cat, horse or human. Effective dosage amounts may vary with the severity of the disease or condition, the route(s) of administration, the duration of the treatment, the identify of any other drugs being administered to the animal, the age, size and species of the animal, and like factors known in the medical and veterinary arts.

Because the treatment of the present invention provides a long-lasting effect on the symptoms of degenerative joint disease, one aspect of the present invention is that the composition comprising DA-DKP may be administered to an animal at longer time intervals than would be expected for conventional therapies. For example, the present composition can be administered no more frequently than once every six month, once every five months, once every four months, once every three months, once every two months, once every month, once every four weeks, once every three weeks, once every two weeks or once every week.

The composition of the present invention comprising DA-DKP may be administered to an animal patient for therapy by any suitable route of administration, including locally, parenterally (e.g., injection, intra-articular injection, intravenously, intraspinally, intraperitoneally, subcutaneously, or intramuscularly), transdermally, and topically. A preferred route of administration is intra-articular injection.

The composition of the present invention may be a pharmaceutical solution having a DA-DKP concentration range with a lower endpoint of about 10 μM, about 20 μM, about 30 μM, about 40 μM, about 50 μM, about 60 μM, about 70 μM, about 80 μM, about 90 μM, about 100 μM, about 110 μM, about 120 μM, about 130 μM, about 140 μM, about 150 μM, about 160 μM, about 170 μM, about 180 μM, about 190 μM, about 200 μM, about 210 μM, about 220 μM, about 230 μM, about 240 μM, about 240, about 250 μM, about 260 μM, about 270 μM, about 280 μM, about 290 μM, about 300 μM, about 310, about 320 μM, about 330 μM, about 340 μM, about 350 μM, about 360 μM, about 370 μM, about 380 μM, about 390 μM, or about 400 μM. The composition of the present invention may be a pharmaceutical solution having a DA-DKP concentration range with an upper endpoint of about 600 μM, about 580 μM, about 570 μM, about 560 μM, about 550 μM, about 540 μM, about 530 μM, about 520 μM, about 510 μM, about 500 μM, about 490 μM, about 480 μM, about 470 μM, about 460 μM, about 450 μM, about 440 μM, about 430 μM, about 420 μM, about 410 μM, about 400 μM, about 390 μM, about 380 μM, about 370 μM, about 360 μM, about 350, about 340 μM, about 330 μM, about

320 μM, about 310 μM, about 300 μM, about 290 μM, about 280, about 270 μM, about 260 μM, about 250 μM, about 240 μM, about 230 μM, about 220 μM, about 210 μM, or about 200 μM.

An effective amount of DA-DKP in the composition of the present invention for treating a degenerative joint disease or condition can be a range with a lower endpoint of about 10 μg, about 15 μg, about 20 μg, about 25 μg, about 30 μg, about 35 μg, about 40 μg, about 45 μg, about 50 μg, about 55 μg, about 60 μg, about 65 μg, about 70 μg, about 75 μg, about 80 μg, about 85 μg, about 90 μg, about 95 μg, about 100 μg, about 110 μg, about 120 μg, about 130 μg, about 140 μg, about 150 μg, about 160 μg, about 170 μg, about 180 μg, about 190 μg, about 200 μg, about 210 μg, about 220 μg, about 230 μg, about 240 μg, about 250 μg, about 260 μg, about 270 μg, about 280 μg, about 290 μg, about 300 μg, about 310 μg, about 320 μg, about 330 μg, about 340 μg, about 350 μg, about 360 μg, about 370 μg, about 380 μg, about 390 μg, about 400 μg, about 425 μg, about 450 μg, about 475 μg or about 500 μg. In addition, an effective amount of DA-DKP in the composition of the present invention for treating a degenerative joint disease or condition can be a range with upper endpoint of about 500 μg, about 490 μg, about 480 μg, about 470 μg, about 460 μg, about 450 μg, about 440 μg, about 430 μg, about 420 μg, about 410 μg, about 400 μg, about 390 μg, about 380 μg, about 370 μg, about 360 μg, about 350 μg, about 340 μg, about 330 μg, about 320 μg, about 310 μg, about 300 μg, about 290 μg, about 280 μg, about 270 μg, about 260 μg, about 250 μg, about 240 μg, about 230 μg, about 220 μg, about 210 μg, about 200 μg, about 190 μg, about 180 μg, about 170 μg, about 160 μg, about 150 μg, about 140 μg, about 130 μg, about 120 μg, about 110 μg, about 100 μg, about 90 μg, about 80 μg, about 70 μg, about 60 μg, about 50 μg, about 40 μg, about 30 μg, or about 20 μg.

Dosage forms for the topical or transdermal administration of compounds of the invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches, and drops. The active ingredient may be mixed under sterile conditions with a pharmaceutically-acceptable carrier, and with any buffers, or propellants which may be required.

The ointments, pastes, creams and gels may contain, in addition to the active ingredient, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid,

talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to the active ingredient, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder or mixtures of these substances. Sprays can additionally contain customary propellants such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

Transdermal patches have the added advantage of providing controlled delivery of compounds of the invention to the body. Such dosage forms can be made by dissolving, dispersing or otherwise incorporating one or more compounds of the invention in a proper medium, such as an elastomeric matrix material. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate of such flux can be controlled by either providing a rate-controlling membrane or dispersing the compound in a polymer matrix or gel.

Pharmaceutical compositions of this invention suitable for parenteral administrations comprise one or more compounds of the invention in combination with one or more pharmaceutically-acceptable sterile isotonic aqueous or non-aqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as wetting agents, emulsifying agents and dispersing agents. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like in the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monosterate and gelatin.

In some cases, in order to prolong the effect of a drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally-administered drug is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissue. The injectable materials can be sterilized for example, by filtration through a bacterial-retaining filter.

The formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a lyophilized condition requiring only the addition of the sterile liquid carrier, for example water for injection, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the type described above.

Kits comprising the pharmaceutical products of the present invention are also provided. The kits can comprise a DA-DKP composition formulated for administration by injection. The DA-DKP can be prepared as described herein, such as by removing albumin from a solution of a human albumin composition. The kits may contain unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a lyophilized condition requiring only the addition of the sterile liquid carrier, for example water for injection, immediately prior to use. The kits may also be stored in a condition, wherein the contents are ready for direct use or injection.

While it is possible for a compound of the present invention to be administered alone, it is preferable to administer the compound as a pharmaceutical formulation (composition). The pharmaceutical compositions of the invention comprise a compound or compounds of the invention as an active ingredient in admixture with one or more

pharmaceutically-acceptable carriers and, optionally, with one or more other compounds, drugs or other materials. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the animal. Pharmaceutically-acceptable carriers are well known in the art. Regardless of the route of administration selected, the compounds of the present invention are formulated into pharmaceutically-acceptable dosage forms by conventional methods known to those of skill in the art. See, e.g., Remington's Pharmaceutical Sciences.

The composition of the present invention may further comprise N-acetyl-tryptophan (NAT), caprylic acid, caprylate or combinations thereof. Preferably, the composition may comprise NAT. Compositions of the present invention having NAT, caprylic acid, caprylate or combinations thereof may be a pharmaceutical composition having a NAT, caprylic acid, caprylate or combinations thereof concentration range with a lower endpoint of about 1 mM, about 2 mM, about 3 mM, about 4 mM, about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, about 10 mM, about 11 mM, about 12 mM, about 13 mM, about 14 mM, about 15 mM, about 16 mM, about 17 mM, about 18 mM, about 19 mM, or about 20 mM. In addition, compositions of the present invention having NAT, caprylic acid, caprylate or combinations thereof may be a pharmaceutical composition having a NAT, caprylic acid, caprylate or combinations thereof concentration range with an upper endpoint of about 40 mM, about 39 mM, about 38 mM, about 37 mM, about 36 mM, about 35 mM, about 34 mM, about 33 mM, about 32 mM, about 31 mM, about 30 mM, about 29 mM, about 28 mM, about 27 mM, about 26 mM, about 25 mM, about 24 mM, about 23 mM, about 22, or about 21 mM. Preferably, the concentration range is about 4 mM to about 20 mM.

In addition, the composition of the present invention may also comprise a second drug such as an analgesic (such as lidocaine or paracetoamol), an anti-inflammatory (such as bethamethasone, non-steroid anti-inflammatory drugs (NSAIDs), acetaminophen, ibuprofen, naproxen), and/or other suitable drugs.

Methods of making diketopiperazines, such as DA-DKP, are well known in the art, and these methods may be employed to synthesize the diketopiperazines of the invention. See, e.g., U.S. Patents Nos. 4,694,081, 5,817,751, 5,990,112, 5,932,579 and 6,555,543, US Patent Application Publication Number 2004/0024180, PCT applications WO 96/00391 and WO 97/48685, and Smith et al., Bioorg. Med. Chem. Letters, 8, 2369-2374 (1998), the

complete disclosures of which are incorporated herein by reference.

For instance, diketopiperazines, such as DA-DKP, can be prepared by first synthesizing dipeptides. The dipeptides can be synthesized by methods well known in the art using L-amino acids, D-amino acids or a combination of D- and L-amino acids. Preferred are solid-phase peptide synthetic methods. Of course, dipeptides are also available commercially from numerous sources, including DMI Synthesis Ltd., Cardiff, UK (custom synthesis), Sigma-Aldrich, St. Louis, MO (primarily custom synthesis), Phoenix Pharmaceuticals, Inc., Belmont, CA (custom synthesis), Fisher Scientific (custom synthesis) and Advanced ChemTech, Louisville, KY.

Once the dipeptide is synthesized or purchased, it is cyclized to form a diketopiperazine. This can be accomplished by a variety of techniques. For example, U.S. Patent Application Publication Number 2004/0024180 describes a method of cyclizing dipeptides. Briefly, the dipeptide is heated in an organic solvent while removing water by distillation. Preferably, the organic solvent is a low-boiling azeotrope with water, such as acetonitrile, allyl alcohol, benzene, benzyl alcohol, n-butanol, 2-butanol, t-butanol, acetic acid butylester, carbon tetrachloride, chlorobenzene chloroform, cyclohexane, 1,2-dichlorethane, diethylacetal, dimethylacetal, acetic acid ethylester, heptane, methylisobutylketone, 3-pentanol, toluene and xylene. The temperature depends on the reaction speed at which the cyclization takes place and on the type of azeotroping agent used. The reaction is preferably carried out at 50-200°C, more preferably 80-150°C. The pH range in which cyclization takes place can be easily determine by the person skilled in the art. It will advantageously be 2-9, preferably 3-7.

When one or both of the amino acids of the dipeptide has, or is derivatized to have, a carboxyl group on its side chain (e.g., aspartic acid or glutamic acid), the dipeptide is preferably cyclized as described in U.S. Patent No. 6,555,543. Briefly, the dipeptide, with the side-chain carboxyl still protected, is heated under neutral conditions. Typically, the dipeptide will be heated at from about 80°C to about 180°C, preferably at about 120°C. The solvent will be a neutral solvent. For instance, the solvent may comprise an alcohol (such as butanol, methanol, ethanol, and higher alcohols, but not phenol) and an azeotropic co-solvent (such as toluene, benzene, or xylene). Preferably, the alcohol is butan-2-ol, and the azeotropic co-solvent is toluene. The heating is continued until the reaction is complete, and such times can be determined empirically. Typically, the dipeptide will be

cyclized by refluxing it for about 8-24 hours, preferably about 18 hours. Finally, the protecting group is removed from the diketopiperazine. In doing so, the use of strong acids (mineral acids, such as sulfuric or hydrochloric acids), strong bases (alkaline bases, such as potassium hydroxide or sodium hydroxide), and strong reducing agents (e.g., lithium aluminum hydride) should be avoided, in order to maintain the chirality of the final compound.

Dipeptides made on solid phase resins can be cyclized and released from the resin in one step. See, e.g., U.S. Patent No. 5,817,751. For instance, the resin having an N-alkylated dipeptide attached is suspended in toluene or toluene/ethanol in the presence of acetic acid (e.g., 1%) or triethylamine (e.g., 4%). Typically, basic cyclization conditions are preferred for their faster cyclization times.

Other methods of cyclizing dipeptides and of making diketopiperazines are known in the art and can be used in the preparation of diketopiperazines useful in the practice of the invention. See, e.g., those references listed above. In addition, many diketopiperazines suitable for use in the present invention can be made as described below from proteins and peptides. Further, diketopiperazines for use in the practice of the invention can be obtained commercially from, e.g., DMI Synthesis Ltd., Cardiff, UK (custom synthesis).

The DA-DKP composition and/or products of the present invention can be prepared from solutions containing DA-DKP, including from the commercially-available pharmaceutical compositions comprising albumin, such as human serum albumin, by well known methods, such as ultrafiltration, chromatography (size-exclusion chromatography (e.g., Centricon filtration), affinity chromatography (e.g., using a column of beads having attached thereto an antibody or antibodies directed to the desired diketopiperazine(s) or an antibody or antibodies directed to the truncated protein or peptide), anion exchange or cation exchange), sucrose gradient centrifugation, chromatography, salt precipitation, or sonication, that will remove some or all of the albumin in the solution. The resultant DA-DKP-containing composition and/or product can be used and incorporated into pharmaceutical compositions as described above.

Using an ultrafilration separation method, a human serum albumin composition can be passed over an ultrafiltration membrane having a molecular weight cut-off that retains the albumin while the DA-DKP passes into the resulting filtrate or fraction. This

filtrate may comprise components having molecular weights less than about 50 kDA, less than about 40 kDa, less than 30 kDa, less than about 20 kDa, less than about 10 kDa, less than about 5 kDa, less than about 3 kDa. Preferably, the filtrate comprises components having molecular weights less than about 5 Da (also referred to as "<5000MW"). This <5000MW fraction or filtrate contains DA-DKP which is formed after the dipeptide aspartate-alanine is cleaved from albumin and subsequently cyclized into the diketopiperazine.

Physiologically-acceptable salts of the DA-DKP of the invention may also be used in the practice of the invention. Physiologically-acceptable salts include conventional non-toxic salts, such as salts derived from inorganic acids (such as hydrochloric, hydrobromic, sulfuric, phosphoric, nitric, and the like), organic acids (such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, glutamic, aspartic, benzoic, salicylic, oxalic, ascorbic acid, and the like) or bases (such as the hydroxide, carbonate or bicarbonate of a pharmaceutically-acceptable metal cation or organic cations derived from N,N-dibenzylethylenediamine, D-glucosamine, or ethylenediamine). The salts are prepared in a conventional manner, e.g., by neutralizing the free base form of the compound with an acid.

As used herein, "a" or "an" means one or more.

As used herein, "comprises" and "comprising" include within their scope all narrower terms, such as "consisting essentially of" and "consisting of" as alternative embodiments of the present invention characterized herein by "comprises" or "comprising". In regard to use of "consisting essentially of", this phrase limits the scope of a claim to the specified steps and materials and those that do not materially affect the basic and novel characteristics of the invention disclosed herein. .

Additional objects, advantages and novel features of the present invention will become apparent to those skilled in the art by consideration of the following non-limiting examples. The following experimental results are provided for purposes of illustration and are not intended to limit the scope of the invention.

EXAMPLES

Example 1

A clinical trial was performed to investigate the effect of intra-articular knee injection of a <5000MW fraction of human serum albumin (also referred to

herein as "Ampion™") for improving joint function and reducing the pain of osteoarthritis of the knee. A randomized, placebo-controlled, double-blind, parallel study with 60 evaluable subjects was chosen as the appropriate design to estimate the treatment effect and safety of the <5000MW Fraction when it was injected into the study knee.

Primary Objective:

To investigate the reduction of pain in subjects with knee osteoarthritis of a single 10 ml intra- articular injection containing the <5000MW Fraction combined with lidocaine/betamethasone suspension compared with the <5000MW Fraction combined with betamethasone suspension or compared with a saline placebo combined with lidocaine/betamethasone suspension.

Secondary Objectives:

To investigate joint function and pain in subjects with knee osteoarthritis, as assessed by the Western Ontario McMaster University Osteoarthritis (WOMAC) Index (Bellamy et al., "Validation study of WOMAC: a health status instrument for measuring clinically important patient relevant outcomes to antirheumatic drug therapy in patients with osteoarthritis of the hip or knee." *J Rheumatology* 1988;15:1833–1840) of a single intra-articular injection of the <5000MW Fraction combined with lidocaine/betamethasone suspension compared with the <5000MW Fraction combined with betamethasone suspension or compared with a saline placebo combined with lidocaine/betamethasone suspension.

To investigate the requirement for rescue medications in subjects with knee osteoarthritis, after a single intra-articular injection of the <5000MW Fraction combined with lidocaine/betamethasone suspension compared with the <5000MW Fraction combined with betamethasone suspension or compared with a saline placebo combined with lidocaine/betamethasone suspension.

To investigate the effect on the range of motion in subjects with knee osteoarthritis and limited range of motion in the study knee due to pain and inflammation, after a single intra- articular injection of the <5000MW Fraction combined with lidocaine/betamethasone suspension compared with the <5000MW Fraction combined with betamethasone suspension or compared with a saline placebo combined with lidocaine/betamethasone suspension.

To compare safety and tolerability between treatments as assessed by reported

adverse events in the study population.

Description of the Protocol

This study was a randomized, placebo-controlled, double-blind, parallel study designed to evaluate the effect of intra-articular knee injection of the <5000MW Fraction in male or female subjects ≥ 40 years old with symptomatic primary knee osteoarthritis for 6 months preceding screening. The study consisted of a 3 week screening period and a 4 day study participation period. Each subject was randomized to receive a single 10 mL knee injection of one of the following:

the <5000MW Fraction combined with lidocaine/betamethasone suspension

the <5000MW Fraction combined with betamethasone suspension

saline placebo combined with lidocaine/betamethasone suspension

Subjects were allowed to leave the clinic following a satisfactory post-dose review by the investigator. Follow-up assessments were performed at 6 hours, 24 hours (Day 2) and 72 hours (Day 4) post-injection. These assessments were conducted at the clinic or externally (e.g. home visit), at the discretion of the investigator.

Duration of Study Participation:

Screening: Between Day – 21 and Day – 1.

Treatment Period: A single injection in the study knee with clinical follow-up over 72 hours, including dosing on Day 1 and follow-up visits on Day 2 and Day 4.

The total duration of study participation was approximately 72 hours for each subject, with a screening interval of up to 21 days.

Study Treatments

Each subject received a single 10 mL injection in one knee of one of the following treatments:

<5000MW Fraction combined with lidocaine/betamethasone suspension

<5000MW Fraction combined with betamethasone suspension

saline placebo combined with lidocaine/betamethasone suspension

Study Population

The study population was 60 patients, male or female, 40-85 years old, fully ambulatory, with symptomatic primary knee osteoarthritis for more than 6 months prior

to screening with Kellgren Lawrence Grade II or III.

Description of Investigational Product

Sterile 2 mL <5000 MW Fraction in rubber stopper storage vials was prepared by Sypharma Pty Ltd., Dandenong, Victoria, Australia. The <5000MW Fraction was combined with either lidocaine/betamethasone suspension or betamethasone suspension in a blinded fashion prior to injection. Saline placebo combined with lidocaine/betamethasone suspension was administered as a third treatment. See Table 1

Table 1 Investigational Product Components:

	<5000MW Fraction	Bethamethasone	Lidocaine	Saline
Treatment A	4 mL	2 mL	4 mL	Nil
Treatment B	4 mL	2 mL	Nil	4 mL
Treatment C	Nil	2 mL	4 mL	4 mL

A single 10 mL injection of study drug combined with lidocaine/betamethasone suspension or combined with betamethasone suspension was injected into the knee joint space under sterile prep conditions using an 18-21 gauge needle.

Study drug was stored at room temperature (59° - 77° F or 15° – 25° C) in a secure area with restricted access.

Randomization and Allocation to Study Treatment

Subjects were allocated to a sequentially numbered treatment in accordance with the randomization schedule following confirmation of eligibility at pre-dose. Each subject was assigned to one of the three treatments, i.e. active <5000MW Fraction (either combined with lidocaine/betamethasone or betamethasone suspension) or saline placebo combined with lidocaine/betamethasone. The allocation of treatment was performed using a block randomization algorithm.

If both knees were osteoarthritic, then at Screening the investigator selected one knee to be the study knee, being the knee that best satisfied the requirements for the study. At the time of dose administration, the study knee received investigational product in accordance with the randomization schedule. The other knee received normal standard of

care.

Efficacy Measures

The range of motion in the study knee was examined by the investigator or nominee for subjects with limited range of motion due to pain and inflammation at pre-dose, 24 and 72 hours post-dose and the global pain assessment (pain numerical rating scale) was performed at pre- dose, 6, 24 and 72 hours post-dose and Western Ontario McMaster University Osteoarthritis (WOMAC) Index 3.1 was completed by subjects at pre-dose, 24 and 72 hours post-dose.

The pain numerical rating scale (NRS) in the study knee was completed at pre-dose, 6 hours post- dose on Day 1, 24 hours post-dose on Day 2 and 72 hours post-dose on Day 4. The pain numerical rating scale was completed prior to WOMAC if the pain scale and WOMAC occur at the same time. The pain NRS is a numerical rating of 0-10, with 0 being no pain, 5 being moderate pain and 10 being worst possible pain.

The range of motion (degrees of flexion and extension) in the study knee was examined by the investigator or nominee for subjects with limited range of motion due to pain and inflammation not by osteophyte growth, at baseline, 24 hours post-dose on Day 2 and 72 hours post-dose on Day 4.

WOMAC Index 3.1 was completed by subjects within 1 hour prior to injection, 24 hours post-dose on Day 2 and 72 hours post-dose on Day 4. The WOMAC Index assesses joint function and pain in subjects with hip or knee osteoarthritis. It measures 24 parameters (questions) with each parameter (question) being rated by the subject on a scale of 1-10. There are three subscores: pain (5 questions); stiffness (2 questions); and function (17 questions).

The data were analyzed using the Student's t-test: mean (SD) difference between treatment groups A and C for the following:

- Mean change in pain NRS at 6 hours, 24 hours and 72 hours (Figure 1);
- Mean change in WOMAC pain subscores at 24 hours and 72 hours (Figures 3A and 3B);
- Mean change in WOMAC function subscores at 24 hours and 72 hours (Figures 5A and 5B); and
- Mean change in WOMAC stiffness subscores at 24 hours and 72 hours (Figures 4A and 4B).

Also, repeated measures ANOVA were calculated: mean (SD) difference between treatment groups A and C for mean change in pain NRS with time (Figure 2).

Results

Results are presented in Figures 1-6, Tables 2 and 3 and are summarized here:

- A trend in improvement in pain NRS with the <5000MW Fraction (Ampion™) was observed. This trend increased with time versus steroids and demonstrates that the effects of the <5000MW Fraction are long lasting.
- WOMAC – no differences were observed in the three subscores analyzed.
- The <5000MW Fraction does not increase adverse events.
- Data on rescue medications and range of motion were only collected in a small percentage of patients and were not analyzed.
- Steroid Potential Time course is shown in Figure 6. The onset of steroids efficacy is rapid: 12-24 hours maximal effect is reached in 1 week and lasts 4 weeks (Ann Rheum Dis 2004; 63:478-482). Short term pain increases from baseline through day 3 and decreases afterwards (Am J Orthop. 2007 Mar; 36(3): 128-31).

Table 2 Baseline Efficacy Variables, mean (SD)

Efficacy Variable	**Ampion™ + Steriod + lidocaine**	**Steroid + lidocane**	**T-test p value**
Pain NRS	4.29 (2.28)	3.61 (1.69)	0.32
WOMAC Pain	4.09 (1.95)	3.99 (2.07)	0.91
WOMAC Stiffness	3.86 (1.78)	3.93 (2.05)	0.54
WOMAC Function	5.15 (2.10)	4.69 (2.26)	0.89

Ampion + Steroid + lidocane: n=20, safety population; n=17, efficacy analysis population
Steroid + lidocane: n=20, safety population; n=18, efficacy analysis population

Table 3 Mean Change in Pain NRS, mean (SD)

Time	Ampion™ + Steriod + lidocaine	Steroid + lidocane	T-test p value
6 hours	-2.31 (2.36)	-2.06 (1.86)	0.73
24 hours	-2.12 (1.58)	-1.61 (2.15)	0.43
72 hours	-2.50 (1.83)	-1.82 (1.74)	0.30

Example 2

A clinical trial was performed to investigate the effect of intra-articular knee injection of the <5000MW Fraction (also referred to herein as "Ampion™") for improving joint function and reducing the pain of osteoarthritis of the knee in adults with symptomatic primary knee osteoarthritis. A randomized, placebo-controlled, double-blind, parallel study with 43 evaluable subjects was chosen as the appropriate design to estimate the treatment effect and safety of the <5000MW Fraction when it was injected into the study knee.

Study Drug

2 arms; each subject received a single 4 ml injection in one knee with one of either Amipon™ or saline.

Study Population

The study population was 43 patient, male or female 40-83 years old (average 63.0, standard deviation (SD) 9.6) 28 were male and 15 were female. All subjects were Caucasian. The subjects' height ranged from 162 to 192 cm (average 175.3, SD 8.1) with weight at screening ranging from 56 to 117 kg (average 88.8, SD 13.89). The subjects were fully ambulatory, with symptomatic primary knee osteoarthritis for more than 6 months prior to screening with Kellgren Lawrence Grade II or III (indicating mild or moderate osteoarthritis). Grade II for 6 subjects and Grade III for 36 subjects. One subject did have Grade IV. If both knees of a subject were osteroarthritic, one knee was selected for study while the other knee received standard of care.

Exclusion Criteria:

The following is the exclusion criteria for the study population:

1. Unfit as a result of medical review and screening investigation
2. A history of allergic reactions to albumin

3. A history of allergic reactions to excipients in 5% human albumin

4. Any intra-articular or local periarticular injection, injection or surgery to the index knee (previous 6 months)

5. Operative arthroscopy (previous 3 months)

6. Surgical procedure to the index knee other than arthroscopy (previous 12 months)

7. Any investigational knee products (previous 12 months)

8. Kellgren Lawrence Grade I or IV (doubtful or severe) osteoarthritis of the knee.

9. Inflammatory or crystal arthropathies, acute fractures, severe loss of bone density, bone necrosis.

10. Isolated patella-femoral syndrome or chondromalacia.

11. Any other disease or condition interfering with the free use and evaluation of the index knee

12. Major injury to the index (previous 12 months)

13. Severe hip osteoarthritis ipsilateral to the index knee.

14. Any pain that could interfere with the assessment of index knee pain

15. Any pharmacological or non-pharmacological treatment started or changed (previous 4 weeks)

16. Use of a. any topical treatment (previous 48 h,) b. All analgesics and NSAIDs except paracetamol (previous 48 h), c. Anticoagulant therapy (previous 48 h) d. Any systemic steroid treatments (previous 14 days), e. All immunosuppressives within a period of 5 times the drug's half life prior to randomization, f. corticosteroids >10 mg prednisolone equivalent per day (previous 30 days), g. Any albumin treatment (previous 3 months)

17. Female subjects who are pregnant or lactating.

18. Female subjects of childbearing potential who have a positive pregnancy test on Day 1 prior to treatment.

Study Assessment

The study consisted of a three week screening period and an 84 day study participation period. Follow-up assessments were performed at 6 hours, 24 hours and 72 hours post injection. Subjects were contact by telephone at Day 8, Day 30 and

Day 84 to evaluate overall pain and mobility and to monitor adverse events. The subjects were offered the option of intra-articular betamethasone injection to the investigative knee for pain relief after Day 8, if deemed necessary following an assessment by the investigator.

Primary Outcome

The pain numerical rating scale (NRS) in the study knee was completed at pre-dose (pre-injection baseline), 6 hours post- dose on Day 1, 24 hours post-dose on Day 2, 72 hours post-dose on Day 4, and at Day 8, Day 30 and Day 84 post-dose (EOS or End-of-study). The pain NRS is a numerical rating of 0-10, with 0 being no pain, 5 being moderate pain and 10 being worst possible pain.

Safety endpoints

The safety endpoints of the study were incidence of adverse events, vital signs at pre-dose and study Day 4, twelve lead ECG readings at screening and 24 hours post-dose, and clinical blood safety tests (biochemical and hematology) assessed at screening and 24 hours post-dose.

Secondary endpoints

The secondary endpoints of the study were percent responders at Day 30 and Day 84, defined as an improvement in pain NRS of 2 or more points, the change from pre-injection baseline in WOMAC Osteoarthritis Index 3.1 (complete scale, pain subscore, stiffness subscore and function subscore) at 24 and 72 hours after intra-articular injection, the change from pre-injection baseline for requirement for rescue medications (paracetamol) to 24 hours and 72 hours after intra-articular injection and changes over time in mobility at Day 8, Day 30 and Day 84 post-dose compared with pre-dose and the immediate post-dose period.

Intent to Treat (ITT) and Safety Population

Study participants who were randomized and received at least one dose of the study medication. ITT refers to subjects that met inclusion/exclusion criteria.

Per protocol Population

Study participants in the ITT set whose pre-dose pain score did not violate inclusion/exclusion criteria.

Efficacy Population

Study participants in the pre-protocol population who did not receive rescue

medication between 8 and 30 days.

Statistical Analyses

Primary: Analysis of covariance (ANCOVA) model to examine the mean (SD) difference between treatment groups for mean change in pain at Day 30 and Day 84 (EOS), adjusted for baseline pain NRS.

Additional: X^2 test for differences in percent responders. Cochran-armitage trend test for differences in clinically significant improvements. Student's t-test: mean (SD) difference in pain NRS at 30 days.

Safety Analysis:

Adverse events and serious adverse events were listed by subject. Summaries were presented by treatment of adverse events classified by MedDRA System Organ Class and Preferred Term, for overall incidence and by severity and relationship to study medication. Incidence of treatment-emergent adverse events were compared between treatment groups. All clinical safety and tolerability data was listed for each subject and summarized by treatment. Vital signs and ECG parameters were tabulated and summarized by treatment. Laboratory values were listed, along with comments as to clinical significance for values outside the laboratory's normal ranges. Changes from screening were assessed for clinical significance.

Results:

Table 4 Population

Analysis Set	Study Size(n)	Ampion™ (n)	Saline (n)
Safety Set	43	22	21
ITT Set	43	22	21
Per-protocol Set[a]	41	20	21
Efficacy evaluable Set[b]	32	17	15

[a]: 2 subjects in the Ampion™ group had baseline pain NRS < 4 points

[b]: 5 subjects in the Ampion™ group and 6 subjects in the saline group required rescue medication

Use of Rescue Medications

Betamethasone injection: there was no apparent difference between the use of betamethasone injections between subjects who received Ampion™ (5 of 22 subjects,

23%) compared with subjects who received saline (6 of 21 subjects, 29%).

Rescue medications (paracetamol): rescue medication for pain relief in the study knee within 24 hours of injection occurred in a similar number of subjects receiving Ampion™ (6 of 22 subjects) compared with subjects receiving saline (6 of 21 subjects), with similar mean doses of paracetamol used in each of the treatment groups.

Efficacy Results:

Table 5 Pain NRS by treatment, mean (SD) pre-protocol population:

Treatment	Pre-dose	6 h Post dose	24 h Post dose	72 h Post dose	Day 8 Post dose	Day 30 Post dose	Day 84 Post dose
Ampion™	4.70 (0.7)	2.00 (1.3)	3.20 (1.5)	2.60 (2.1)	2.90 (2.1)	2.90 (1.8)	3.21 (1.8)
Saline	5.29 (1.4)	2.67 (1.9)	3.00 (1.7)	2.86 (2.1)	3.33 (1.9)	3.86 (2.2)	4.81 (2.3)

Table 6 Least Squares (LS) Mean Change in Pain NRS: per-protocol population

Treatment	6 h Post dose	24 h Post dose	72 h Post dose	Day 8 Post dose	Day 30 Post dose	Day 84 Post dose
D (Ampion™)	-3.06	-1.69	-2.31	-2.00	-2.16	-1.60
E (Saline)	-2.28	-2.11	-2.22	-1.76	-1.09	-0.36
P value	0.15	0.42	0.89	0.71	0.12	0.07

Scale: -10 =largest possible improvement in pain from baseline, 10 = smallest possible improvement (largest increase) in pain from baseline.
Day 1: 6 hours post-dose
*adjusted for baseline pain NRS

Table 7 LS Mean Change in Pain NRS: efficacy evaluable population

Treatment	6 h Post dose	24 h Post dose	72 h Post dose	Day 8 Post dose	Day 30 Post dose	Day 84 Post dose
D (Ampion™)	-2.91	-1.99	-2.94	-2.45	-2.29	-2.22
E (Saline)	-2.62	-2.61	-2.79	-2.22	-1.17	-0.46
P value	0.62	0.19	0.79	0.71	0.19	0.04

Scale: -10 =largest possible improvement in pain from baseline, 10 = smallest possible improvement (largest increase) in pain from baseline.
Day 1: 6 hours post-dose
*adjusted for baseline pain NRS

Percent responders at Day 84 (EOS): per-protocol population (see Table 8)

Responder: decrease in Day 84 pain NRS of -2 to -10 points (with -10 being the largest possible improvement in pain).

Non-responder: decrease in pain at Day 84 of -1 to 10 (with 10 being the largest possible increase in pain).

Table 8 Trends in pain at 30 days from baseline, by treatment group:

Treatment	Non-Responder	Responder	P value
Ampion™	47.4%	52.6%	0.06
Saline	76.2%	23.8%	

Percent responders at Day 84 (EOS): efficacy evaluable population (see Table 9)

Responder: decrease in Day 84 pain NRS of -2 to -10 points (with -10 being the largest possible improvement in pain).

Non-responder: decrease in pain at Day 84 of -1 to 10 points (with 10 being the largest possible increase in pain).

Table 9 Trends in pain at 30 days from baseline, by treatment group:

Treatment	Non-Responder	Responder	P value
Ampion™	35.7%	64.3%	0.10
Saline	66.7%	33.3%	

Summary of findings: Efficacy:

Overall pain (as assessed by the pain numerical rating score) and WOMAC scores

were reduced post-dose for each of the treatment groups for the duration of the study ($p < 0.05$), except placebo at Day 84. In addition, there was a trend in a significant difference between changes from baseline at Day 30 and at Day 84 for subjects who received Ampion™ compared to subjects who received saline placebo (Day 30: $p = 0.12$; Day 84: $p = 0.07$). This trend became statistically significant in subjects who did not receive rescue medication ($p = 0.04$). There was a trend towards a higher percentage of responders at the end of the study (Day 84) for subjects receiving Ampion™ vs. Placebo ($p = 0.06$). Use of paracetamol rescue medication up to 72 hours post-dose was highest in the Treatment E group (saline). See Figure 7.

Adverse Events (AEs)

Treatment-emergent AEs were reported for 20 of the 43 subjects (47%) following dose administration, with a total of 27 AEs. Commonly occurring AEs were headache and joint swelling and stiffness in the knee. Most subjects reported AEs classified as mild only (16 of 43 subjects, 37%). Only 4 subjects (9%) reported AEs of moderate severity:

- Ampion™: Joint injury and hypertension
- Saline: Back pain and vessel puncture site haematoma

There were no apparent differences in the incidence of moderate AEs between subjects who received Ampion™ (2 subjects, 9%) compared with subjects who received saline (2 subjects, 10%). These AEs were all deemed to be probably not or definitely not related to study drug.

There were no AEs classified as severe.

AEs deemed to be related to study drug administration (possibly) were reported in 3 of 43 subjects (7%). There were no apparent differences in the incidence of related AEs between subjects who received Ampion™ (1 subject, 5%) compared with subjects who received saline (2 subjects, 10%):

a. Headache of mild severity which commenced 5 minutes after treatment administration and resolved 1.8 hours later (Ampion™)
b. Headache of mild severity which commenced 5 hours after treatment administration and resolved 0.5 hours later (saline)
c. Joint swelling of right knee (study knee) of mild severity which commenced 2.4 days after treatment administration and resolved 21 hours later (saline)

Overall, a higher proportion of treatment-emergent AEs were reported in subjects

who received saline (12 subjects, 57%) compared with subjects who received Ampion™ (8 subjects, 36%). AEs deemed to be related to study drug administration (possibly) were reported in 3 of 43 subjects (7%) and included headache and joint swelling of the knee. There were no deaths or other serious AEs. There were no clear differences in safety as assessed by biochemistry clinical laboratory tests, vital signs, and ECG assessments between treatments.

Conclusions of study:

Pain (as assessed by the pain numerical rating score) and WOMAC scores were reduced post-dose for each of the treatment groups for the duration of the study, except placebo at Day 84, with no significant differences between treatment groups. Despite a higher baseline pain NRS for the saline group compared to the Ampion™ group, there was a trend towards a long-term effect of study drug, with a higher percentage of subjects who responded at Day 84 for Ampion™ compared to saline. In subjects receiving Ampion™, overall pain was reduced post-dose for the duration of the study, whereas subjects receiving saline did not have a reduction in pain post-dose at Day 84. Use of paracetamol rescue medication up to 72 hours post-dose was highest in the Treatment E group (saline). Ampion™ was considered safe and well tolerated at the dose used in the study.

Example 3

This study demonstrates that DA-DKP and N-Acetyl Tryptophan (NAT) of the <5kDa fraction of 5% HAS binds to collagen. In particular, at equimolar concentration, NAT binds more to oxidized collagen IV than DA-DKP.

Methods

Collagen source: 1 mg/ml Collagen IV form human placenta in 0.25% acetic acid (Sigma™). The collagen was oxidixed with 500 μM H2O2 in 1XPBS for 1 hour at 37°C. The oxidized collagen IV (final = 0.2 mg/ml) was then incubated with 2 μM DA-DKP or 2 μM NAT in PBS for 1 hour at 37°C. At the end of the incubation, the <5kDa fraction was isolated by a Vivaspin 2 microcentrifugal filter having a molecular weight cutoff of 5,000. The <5kDa fraction was analyzed for DA-DKP and/or NAT content using a liquid chromatograph-mass spectrometery (LCMS) method for DA-DKP analysis.

The results of the analyses are shown in Table 10.

Table 10 Potential Binding of NAT and DA-DKP to H2O2 treated Human Collagen

Sample	Average % decrease	Standard Deviation
2 μM DA-DKP + Collagen IV	26.0%	4.2%
2 μM DA-DKP + Collagen IV w/ 2 μM NAT	25.6%	5.7%
2 μM NAT + Collagen IV	48.9%	1.3%
2 μM NAT + Collagen IV w/ 2 μM DA-DKP	28.7%	9.4%

The results provided in Table 10 demonstrate that DA-DKP and NAT bind to oxidized forms of collagen and hence block the oxidized sites from triggering immune cell activation.

While various embodiments of the present invention have been described in detail, it is apparent that modifications and adaptations of those embodiments will occur to those skilled in the art. It is to be expressly understood, however, that such modifications and adaptations are within the scope of the present invention, as set forth in the following exemplary claims.

WHAT IS CLAIMED:

1. A method of treating a degenerative joint disease comprising administering to an animal in need thereof an effective amount of a pharmaceutical composition comprising DA-DKP.
2. The method of Claim 1, wherein the degenerative joint disease is osteoarthritis.
3. The method of Claim 1 wherein the composition is administered no more frequently than once every six months, once every 5 months, once every 4 months, once every 3 months, or once every 2 months.
4. The method of Claim 1, wherein the composition is administered by an administration route selected from the group consisting of local administration, topical administration, and injection.
5. The method of Claim 4, wherein administration by injection is by intra-articular injection.
6. The method of Claim 5, wherein the composition administered by intra-articular injection is a composition having a concentration of DA-DKP from about 50 μM to about 350 μM.
7. The method of Claim 1, wherein the composition further comprises N-acetyl-tryptophan (NAT), caprylic acid, caprylate or combinations thereof.
8. The method of Claim 7, wherein the composition is a composition having a concentration of NAT, caprylic acid, caprylate or combinations thereof from about 4 mM to about 20 mM.
9. The method of Claim 1, wherein the DA-DKP is in a composition prepared by removing albumin from a solution of a human serum albumin composition.
10. The method of Claim 9, wherein the step of removing the albumin comprises treating the human serum albumin composition by a separation method selected from the group consisting of ultrafiltration, sucrose gradient centrifugation, chromatography, salt precipitation, and sonication.
11. The method of Claim 10, wherein the step of removing comprises passing the human serum albumin composition over an ultrafiltration membrane with a molecular weight cut off that retains the albumin, and wherein the resulting filtrate comprises DA-DKP.

12. The method of Claim 11, wherein the ultrafiltration membrane has a molecular weight cutoff of less than 50 kDa, less than 40 kDa, less than 30 kDa, less than 20 kDa, less than 10 kDa, less than 5 kDa or less than 3 kDa.

13. The method of Claim 9, wherein the composition further comprises NAT, caprylic acid, caprylate or combinations thereof.

14. The method of Claim 13, wherein the composition is a composition having a concentration of NAT, caprylic acid, caprylate or combinations thereof from about 4 mM to about 20 mM.

15. The method of Claim 1, wherein the pharmaceutical composition further comprises a second drug selected from the group consisting of an analgesic, an anti-inflammatory drug, and combinations thereof.

16. A pharmaceutical product comprising a DA-DKP-containing composition formulated for administration by injection.

17. The pharmaceutical product of Claim 16, wherein the product is formulated for administration by intra-articular injection.

18. The pharmaceutical product of claim 16, wherein the DA-DKP is prepared by removing albumin from a solution of a human serum albumin composition.

19. The pharmaceutical product of Claim 18, wherein the step of removing the albumin comprises treating the human serum albumin composition by a separation method selected from the group consisting of ultrafiltration, sucrose gradient centrifugation, chromatography, salt precipitation, and sonication.

20. The pharmaceutical product of Claim 19, wherein the step of removing comprises passing the human serum albumin composition over an ultrafiltration membrane with a molecular weight cut off that retains the albumin, and wherein the resulting filtrate comprises DA-DKP.

21. The pharmaceutical product of Claim 20, wherein the ultrafiltration membrane has a molecular weight cutoff of less than 50 kDa, less than 40 kDa, less than 30 kDa, less than 20 kDa, less than 10 kDa, less than 5 kDa or less than 3 kDa.

22. The pharmaceutical product of Claim 17, wherein the DA-DKP administered by intra-articular injection is a composition having a concentration of DA-DKP from about 50 μM to about 350 μM.

23. The pharmaceutical product Claim 16, wherein the DA-DKP composition further comprises NAT, caprylic acid, caprylate and combinations thereof.

24. The pharmaceutical product of Claim 23, wherein the composition is a composition having a concentration of NAT, caprylic acid, caprylate or combinations thereof from about 4 mM to about 20 mM.

25. The pharmaceutical product of Claim 16, further comprising a pharmaceutically-acceptable carrier.

26. A kit comprising a pharmaceutical product comprising a DA-DKP-containing composition formulated for administration by injection.

27. The kit of Claim 26, wherein the DA-DKP is prepared by removing albumin from a solution of a human serum albumin composition.

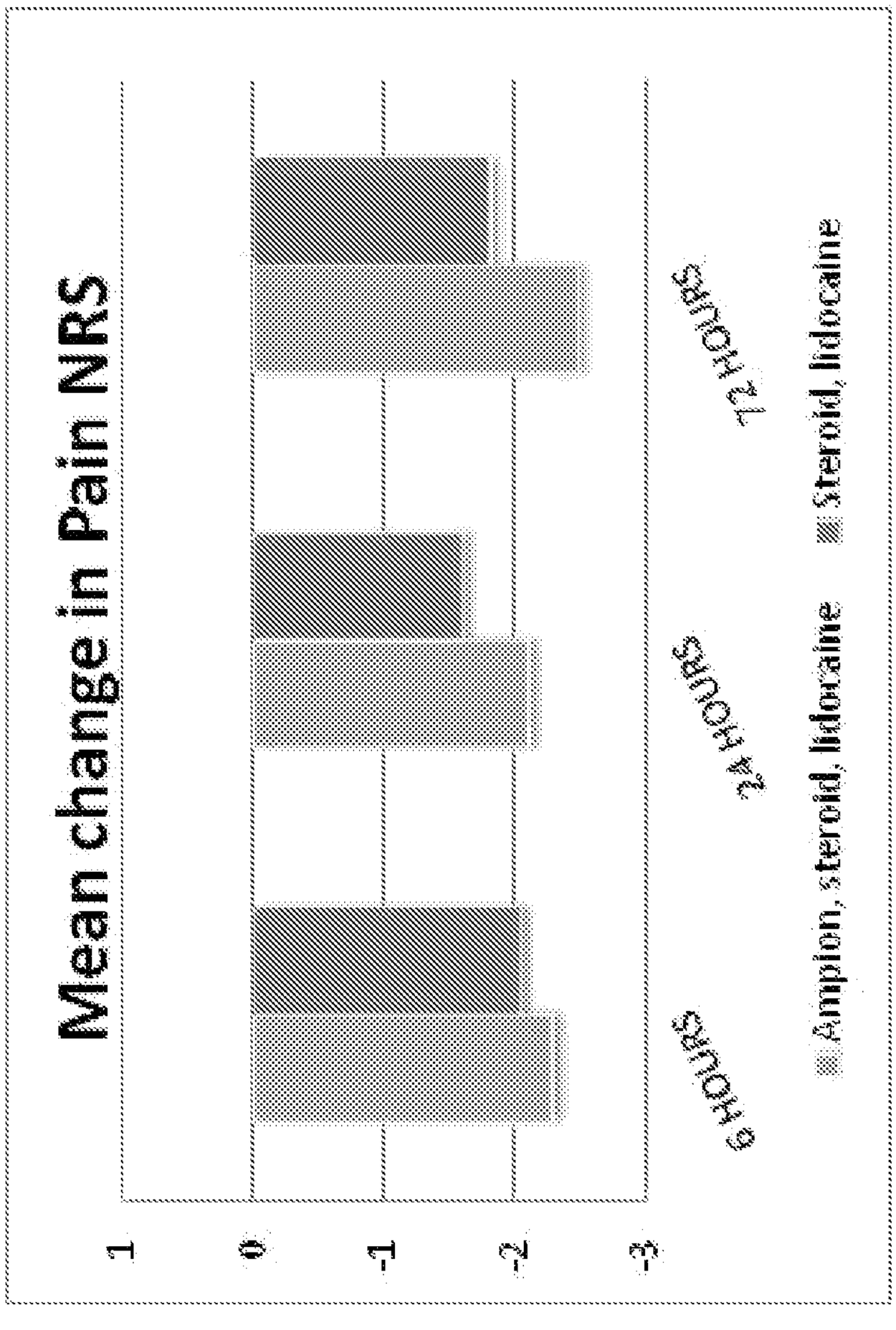

Mean change in Pain NRS
1
0
-1
-2
-3
6 hours
24 hours
6 hours
24 hours
Ampion, steroid, lidocaine
Steroid, lidocaine

Figure 1

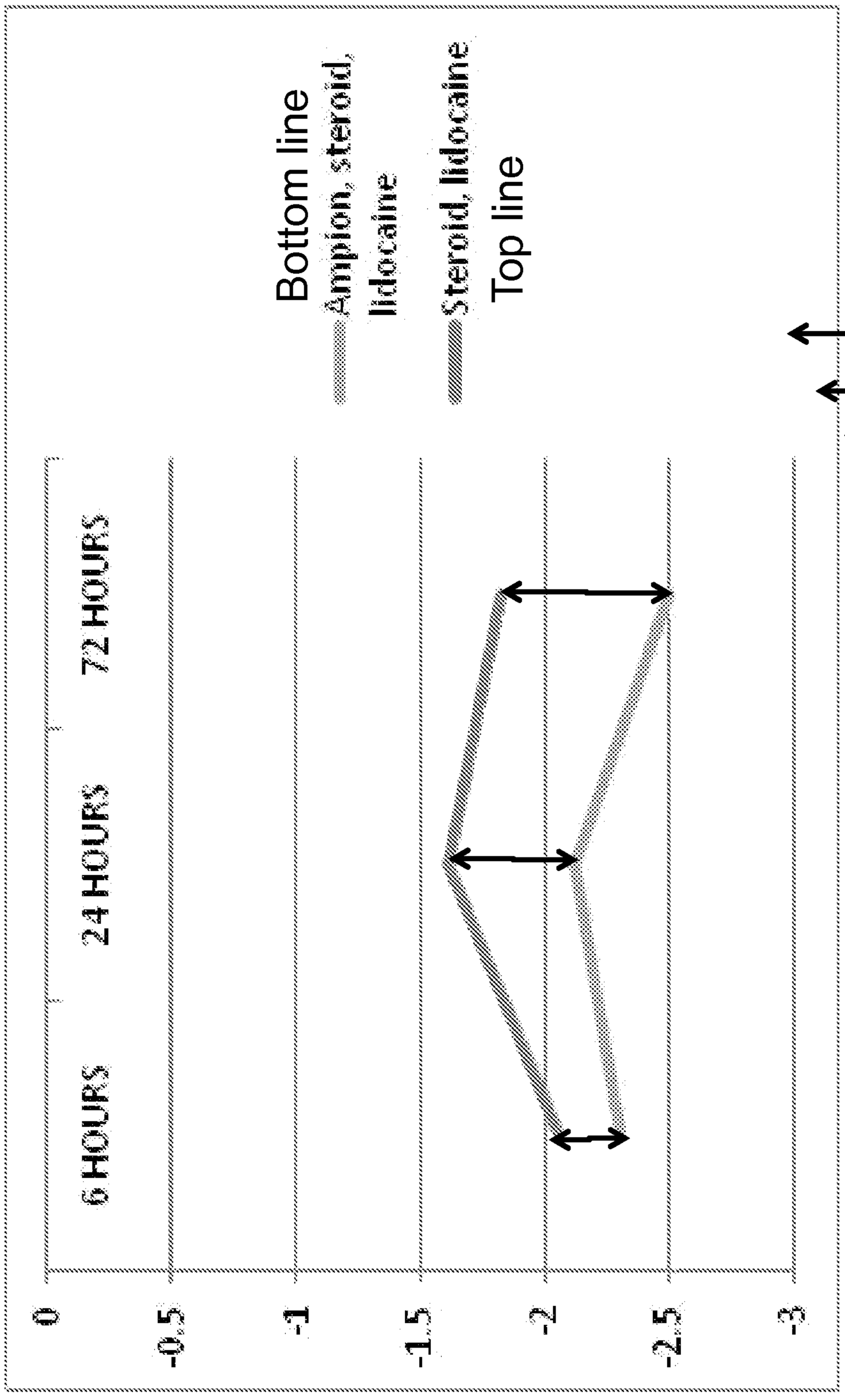


Figure 2
Mean change in pain NRS
Bottom line
Ampion, steroid
Lidocaine
Steroid, lidocaine
Top line
6 HOURS
24 HOURS
72 HOURS
0
-0.5
-1
-1.5
-2
-2.5
-3
• Repeated measures ANOVA p value = 0.30

Figure 3A

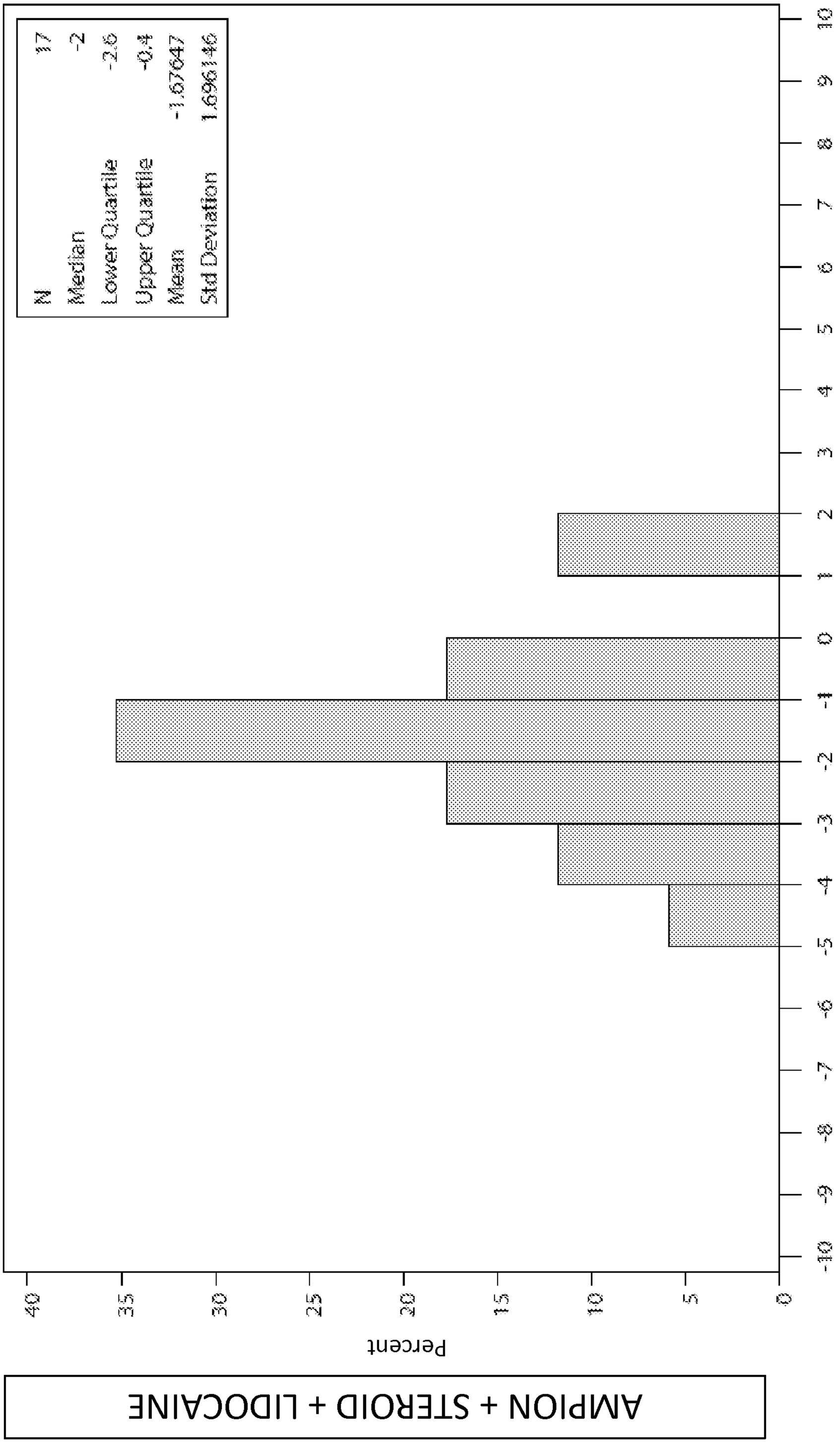

ABSOLUTE DIFFERENCE IN WOMAC PAIN FROM BASELINE
24 HOURS-BASELINE
N 17
Median -2
Lower Quartile -2.6
Upper Quartile -0.4
Mean -1.67647
Std Deviation 1.696146
10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3 -4 -5 -6 -7 -8 -9 -10
40 35 30 25 20 15 10 5 0
Percent
AMPION + STEROID + LIDOCAINE

Figure 3A Cont.

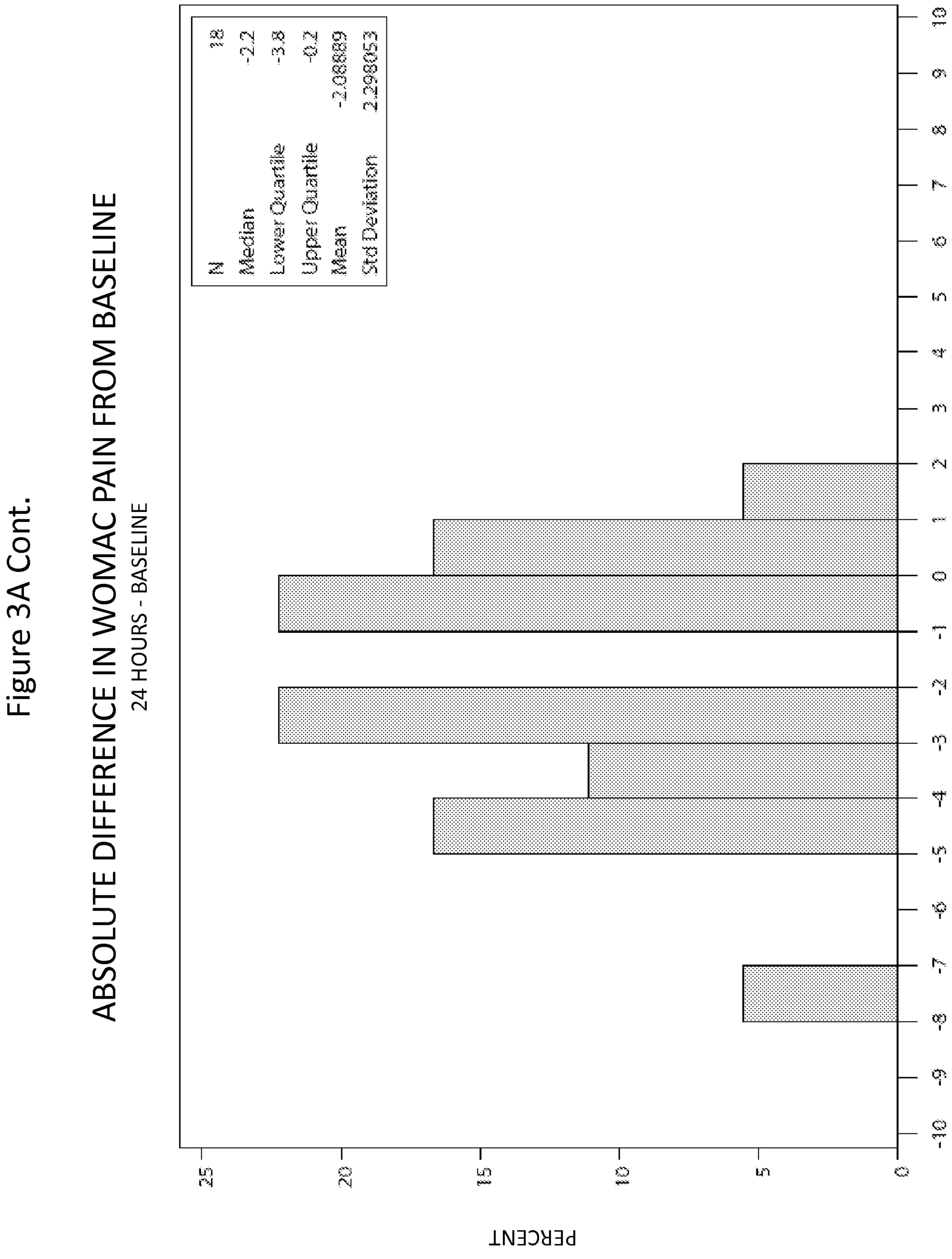


Figure 3B

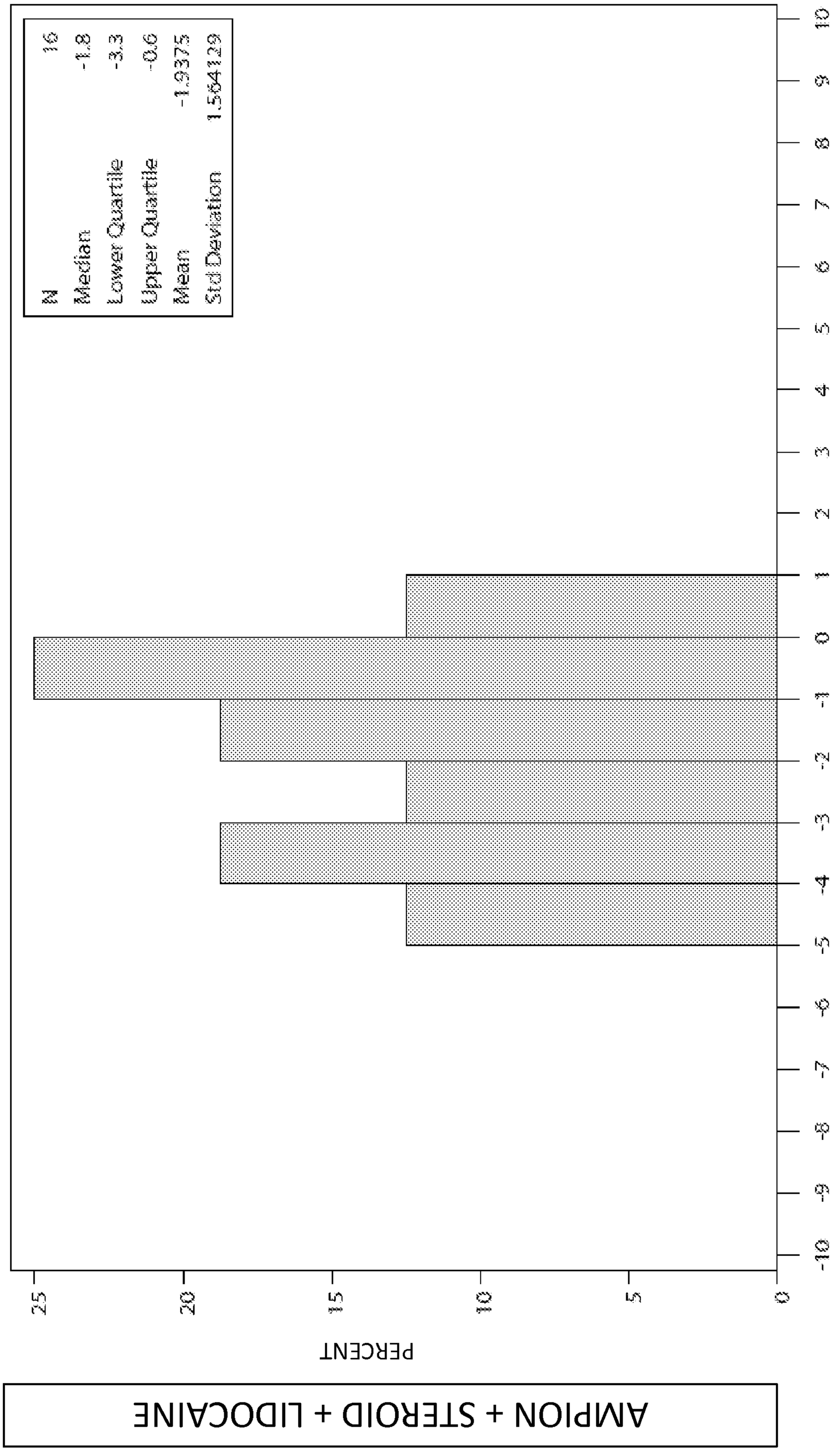

ABSOLUTE DIFFERENCE IN WOMAC PAIN FROM BASELINE
72 HOURS - BASELINE
N 16
Median -1.8
Lower Quartile -3.3
Upper Quartile -0.6
Mean -1.9375
Std Deviation 1.564129
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
25
20
15
10
5
0
PERCENT
AMPION + STEROID + LIDOCAINE

Figure 3B Cont.

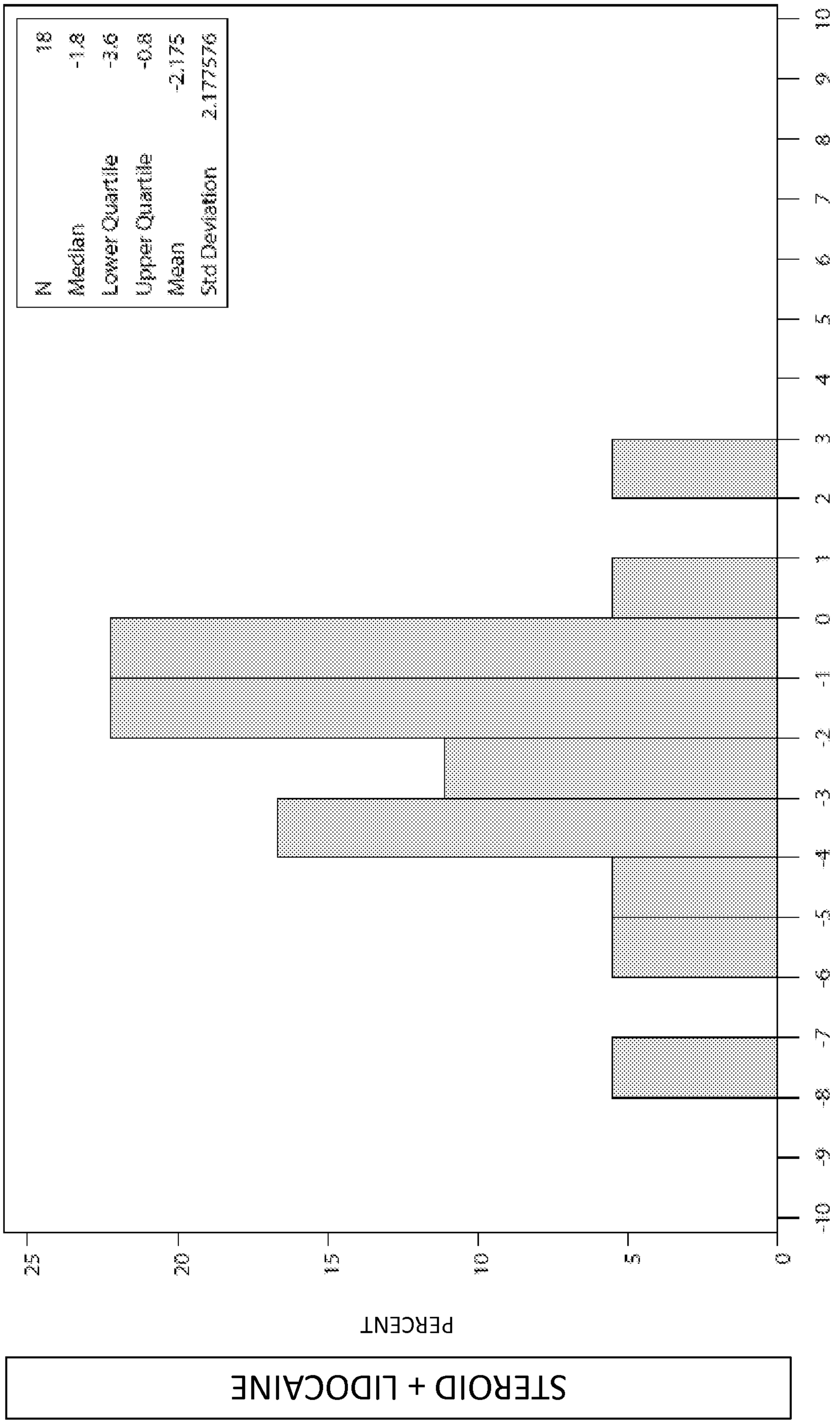


Figure 4A

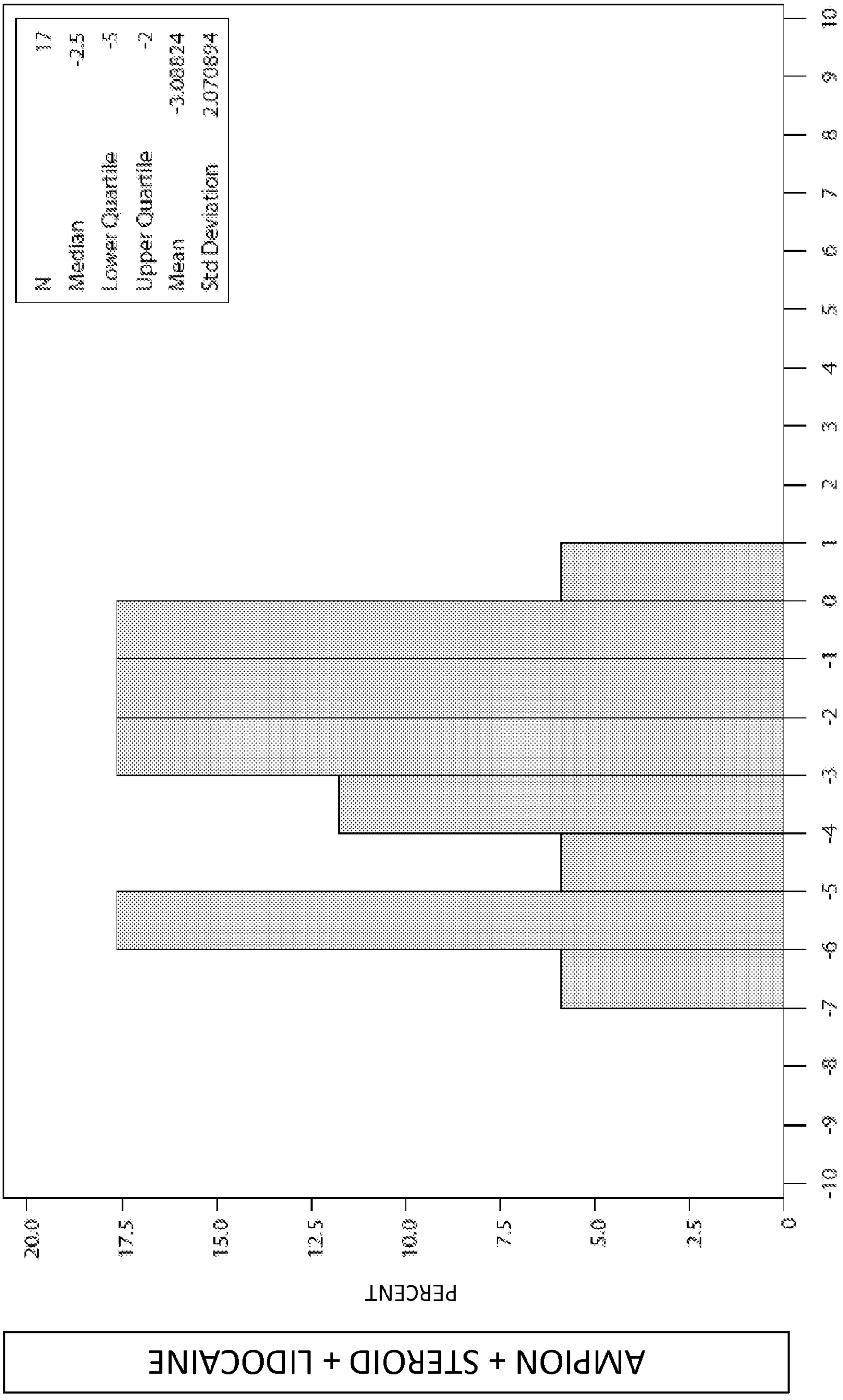

ABSOLUTE DIFFERENCE IN WOMAC STIFFNESS FROM BASELINE
24 HOURS - BASELINE
N 17
Median -2.5
Lower Quartile -5
Upper Quartile -2
Mean -3.08824
Std Deviation 2.070894
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
20.0
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0
PERCENT
AMPION + STEROID + LIDOCAINE

Figure 4A Cont.

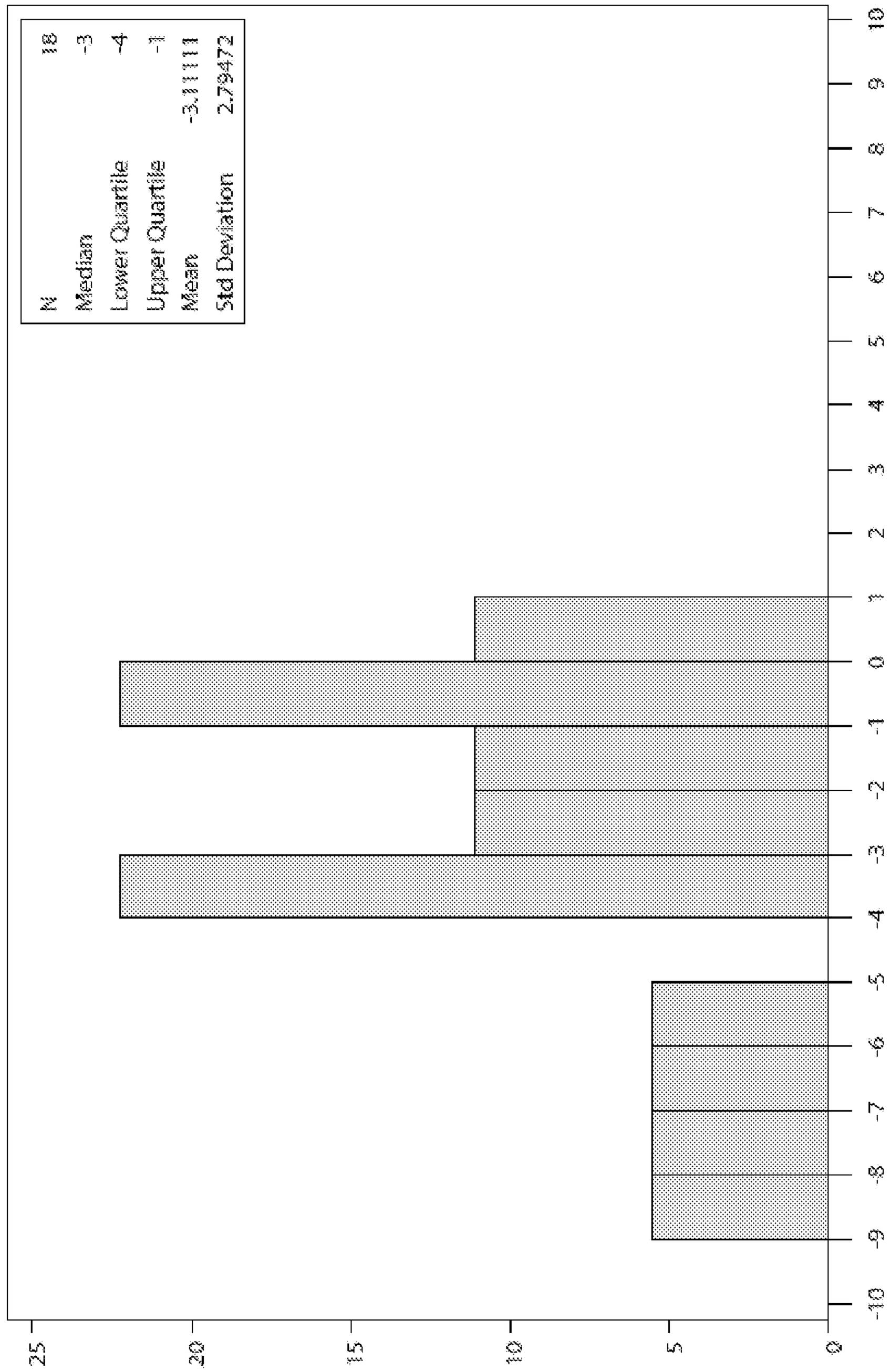


Figure 4B

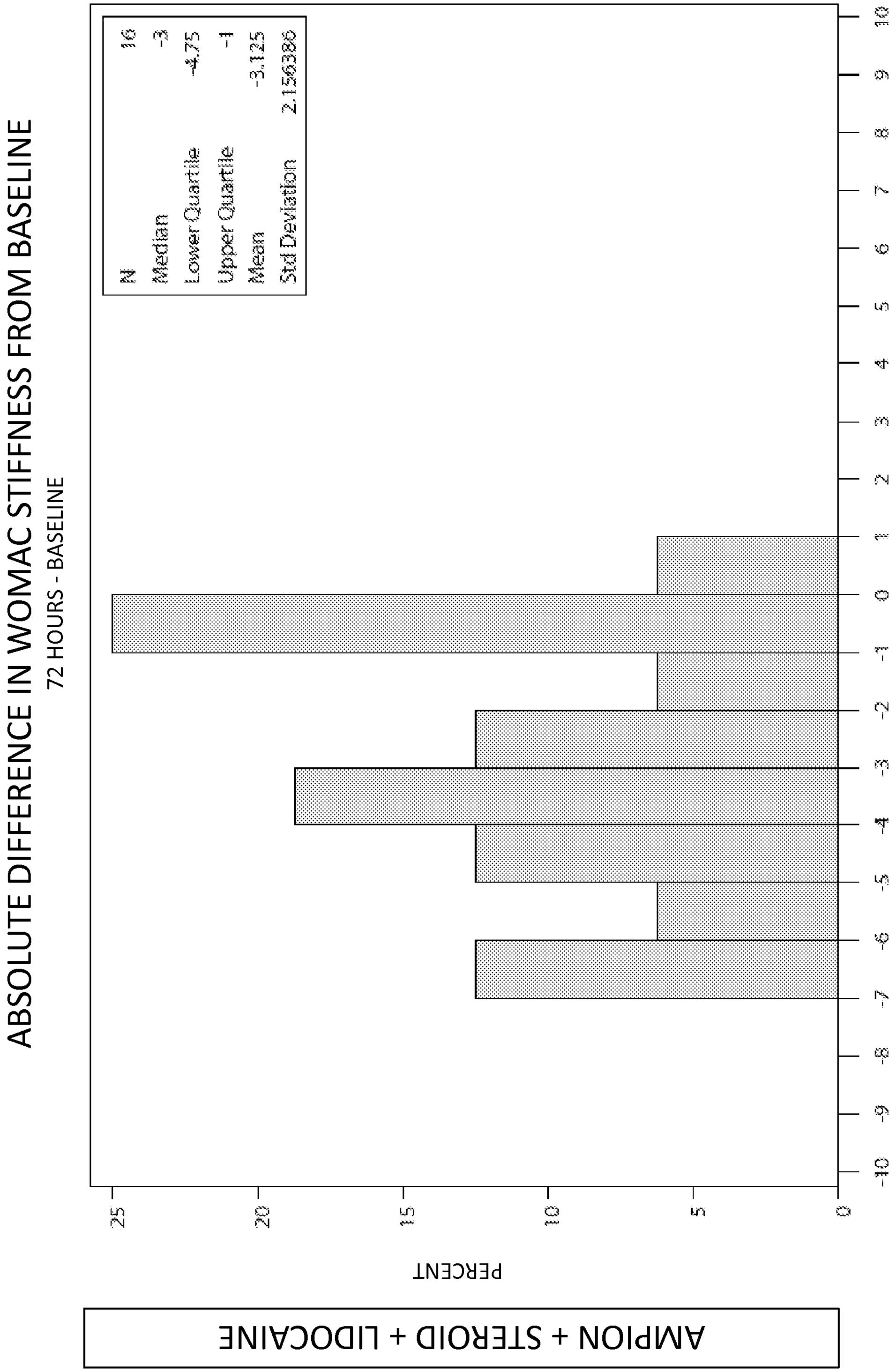

ABSOLUTE DIFFERENCE IN WOMAC STIFFNESS FROM BASELINE
72 HOURS - BASELINE
N 16
Median -3
Lower Quartile -4.75
Upper Quartile -1
Mean -3.125
Std Deviation 2.156386
10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3 -4 -5 -6 -7 -8 -9 -10
25 20 15 10 5 0
PERCENT
AMPION + STEROID + LIDOCAINE

Figure 4B Cont.

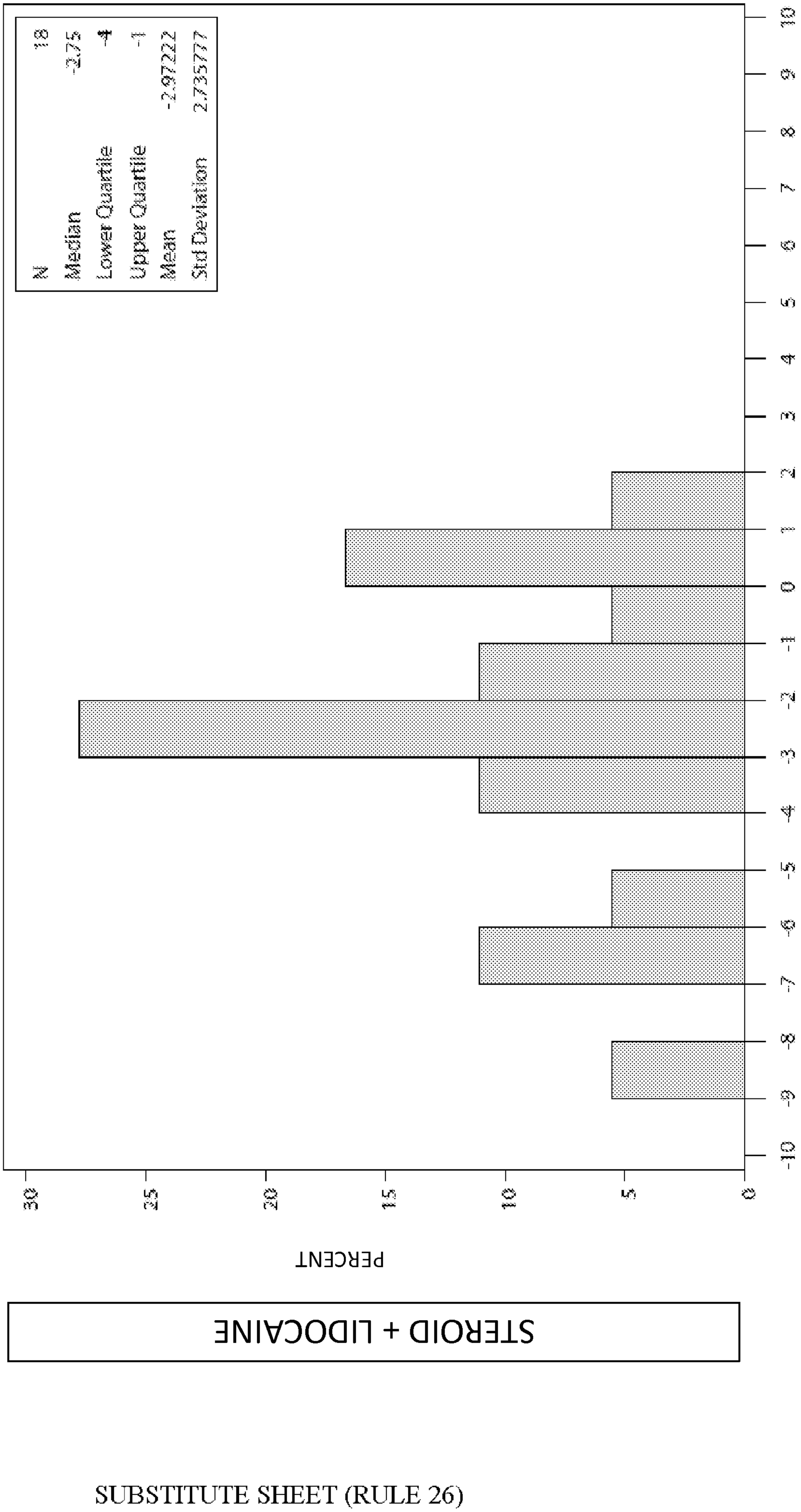


Figure 5A

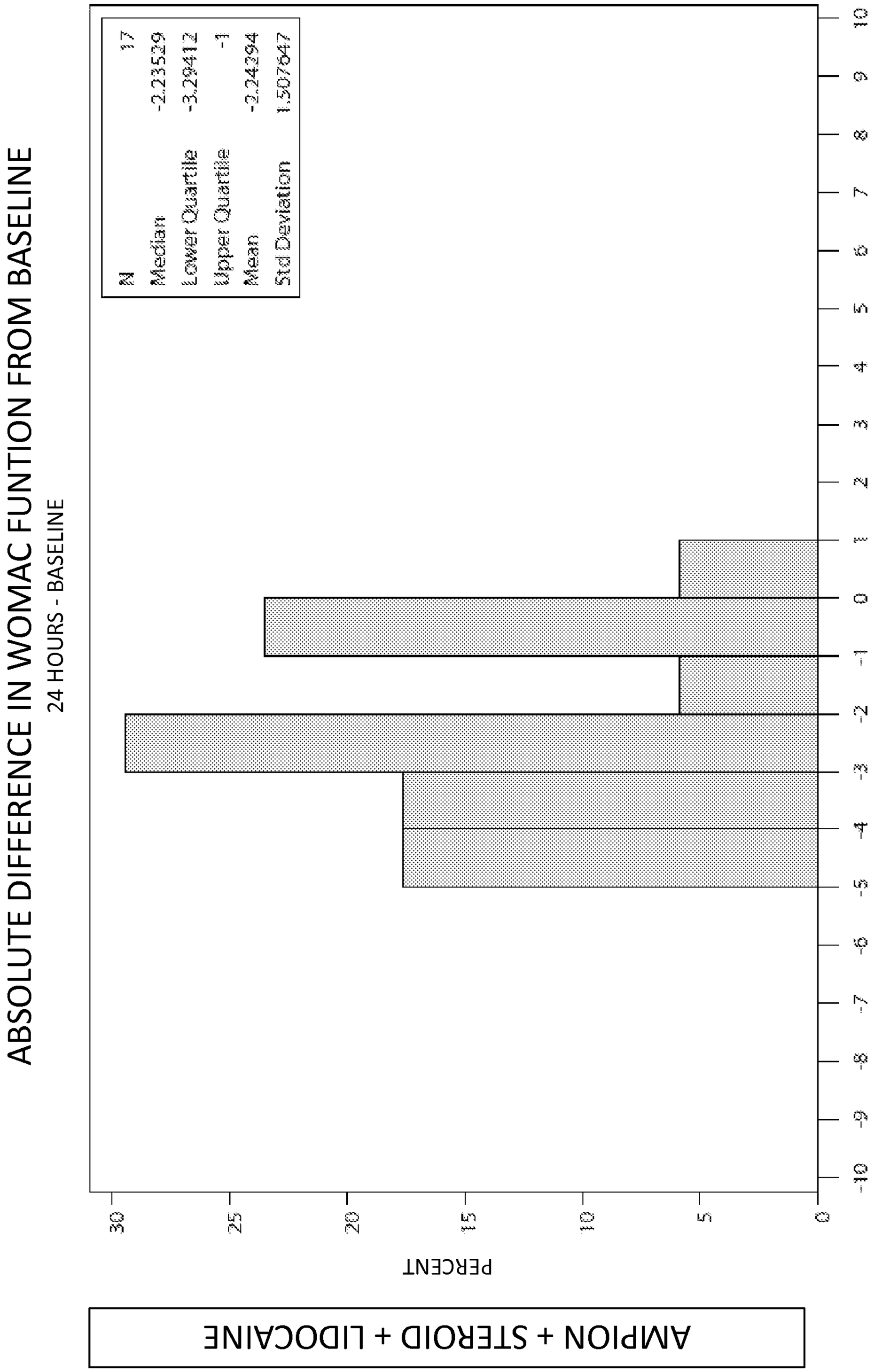

ABSOLUTE DIFFERENCE IN WOMAC FUNTION FROM BASELINE
24 HOURS - BASELINE
N 17
Median -2.23529
Lower Quartile -3.29412
Upper Quartile -1
Mean -2.24394
Std Deviation 1.507647
10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3 -4 -5 -6 -7 -8 -9 -10
30 25 20 15 10 5 0
PERCENT
AMPION + STEROID + LIDOCAINE

Figure 5A Cont.

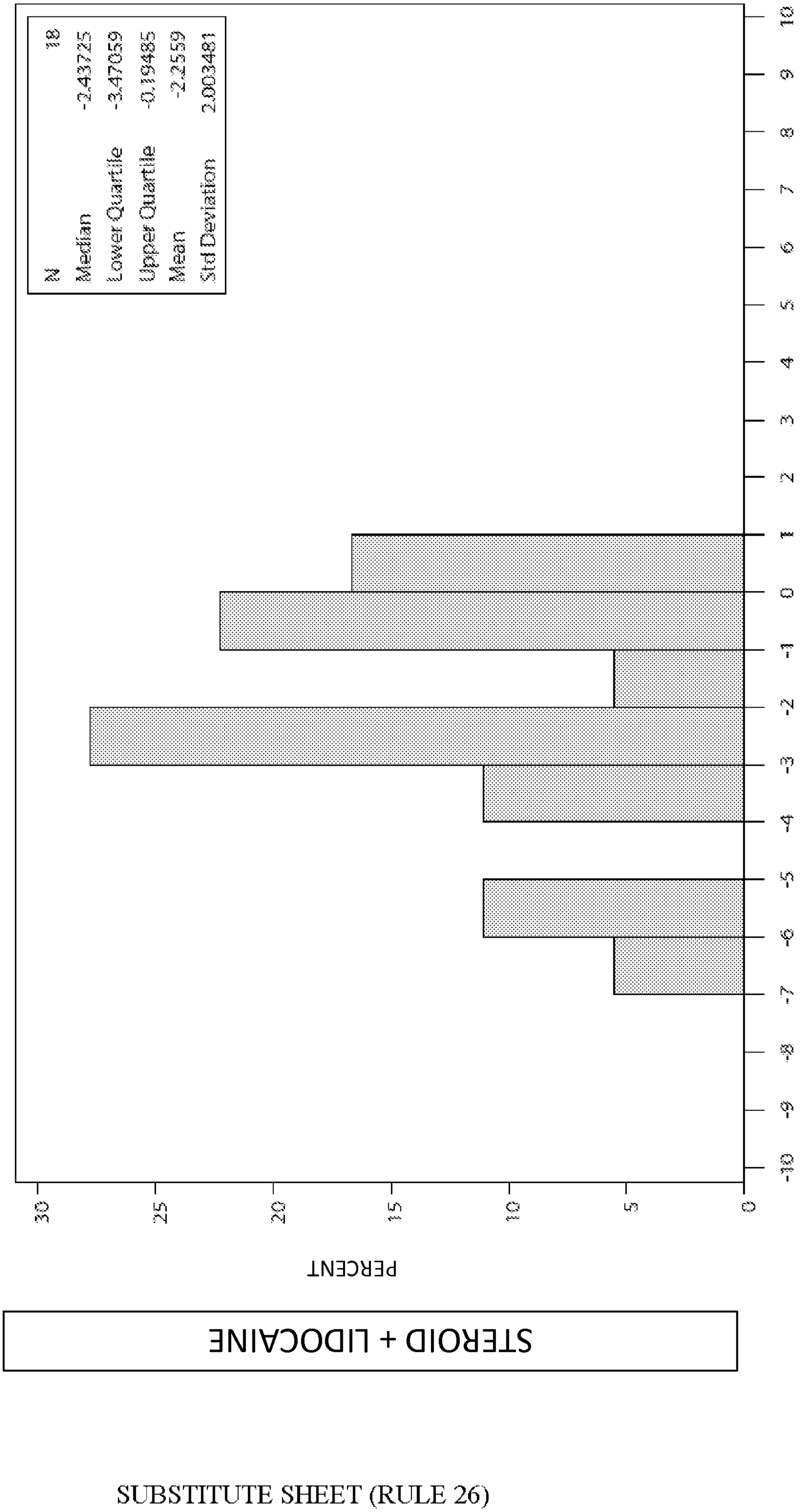


Figure 5B

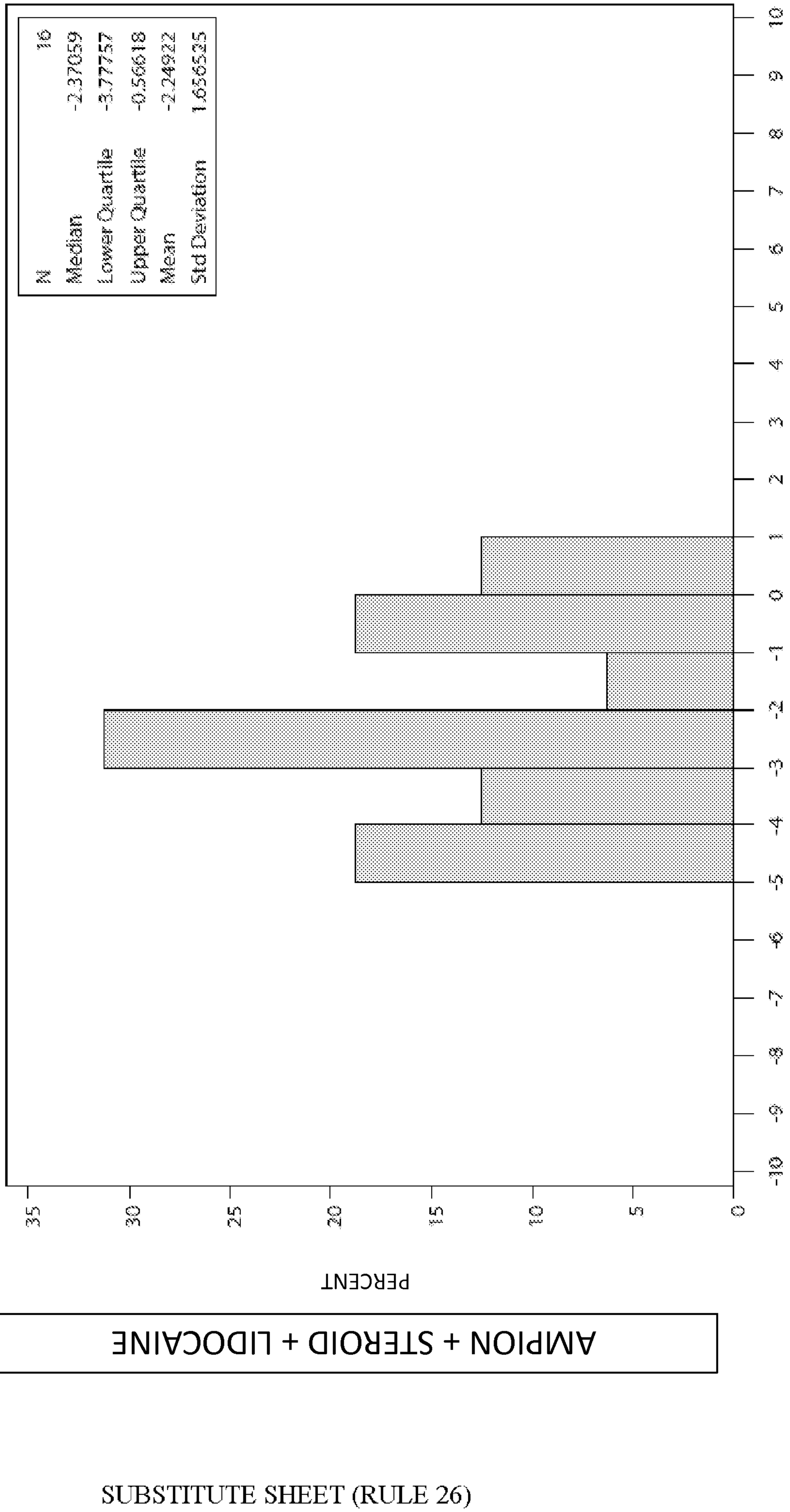

ABSOLUTE DIFFERENCE IN WOMAC FUNCTION FROM BASELINE
72 HOURS - BASELINE
N 16
Median -2.37059
Lower Quartile -3.77757
Upper Quartile -0.56618
Mean -2.24922
Std Deviation 1.6565335
10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3 -4 -5 -6 -7 -8 -9 -10
35 30 25 20 15 10 5 0
PERCENT
AMPION + STEROID + LIDOCAINE

Figure 5B Cont.

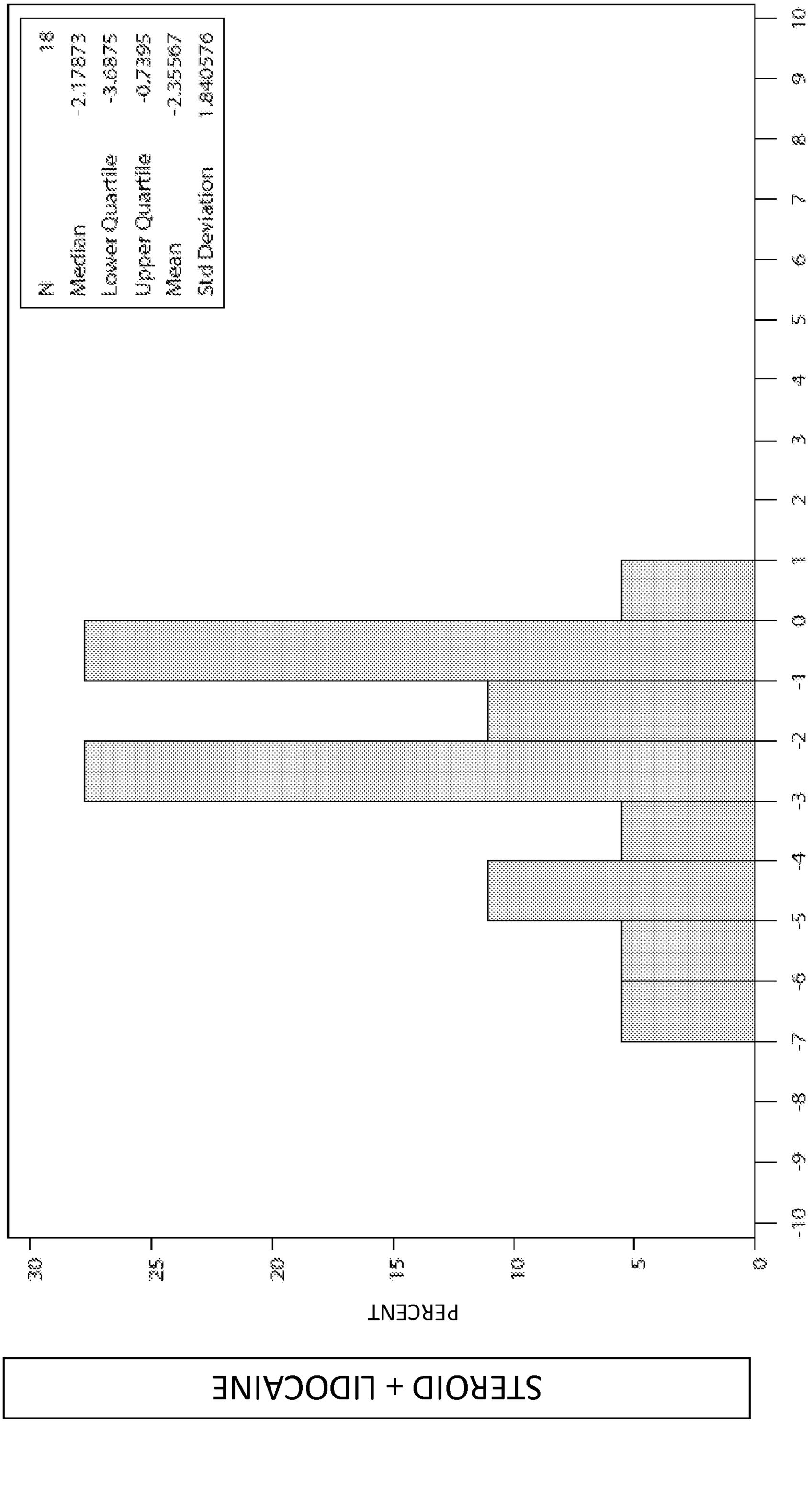
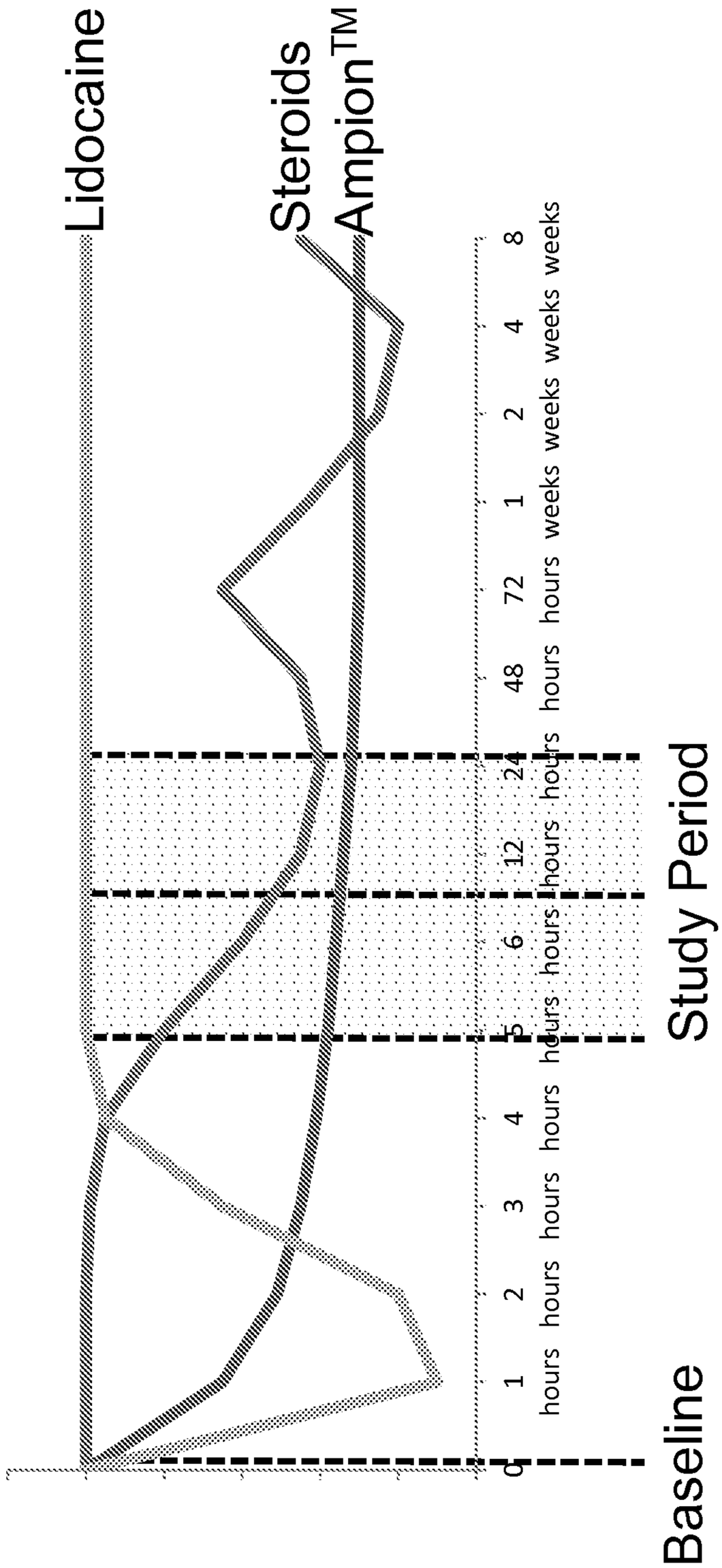


Figure 6


Lidocaine
Steroids
Ampion™
Baseline
Study Period
1 hours
2 hours
3 hours
4 hours
5 hours
6 hours
12 hours
24 hours
48 hours
72 hours
1 weeks
2 weeks
4 weeks
8 weeks

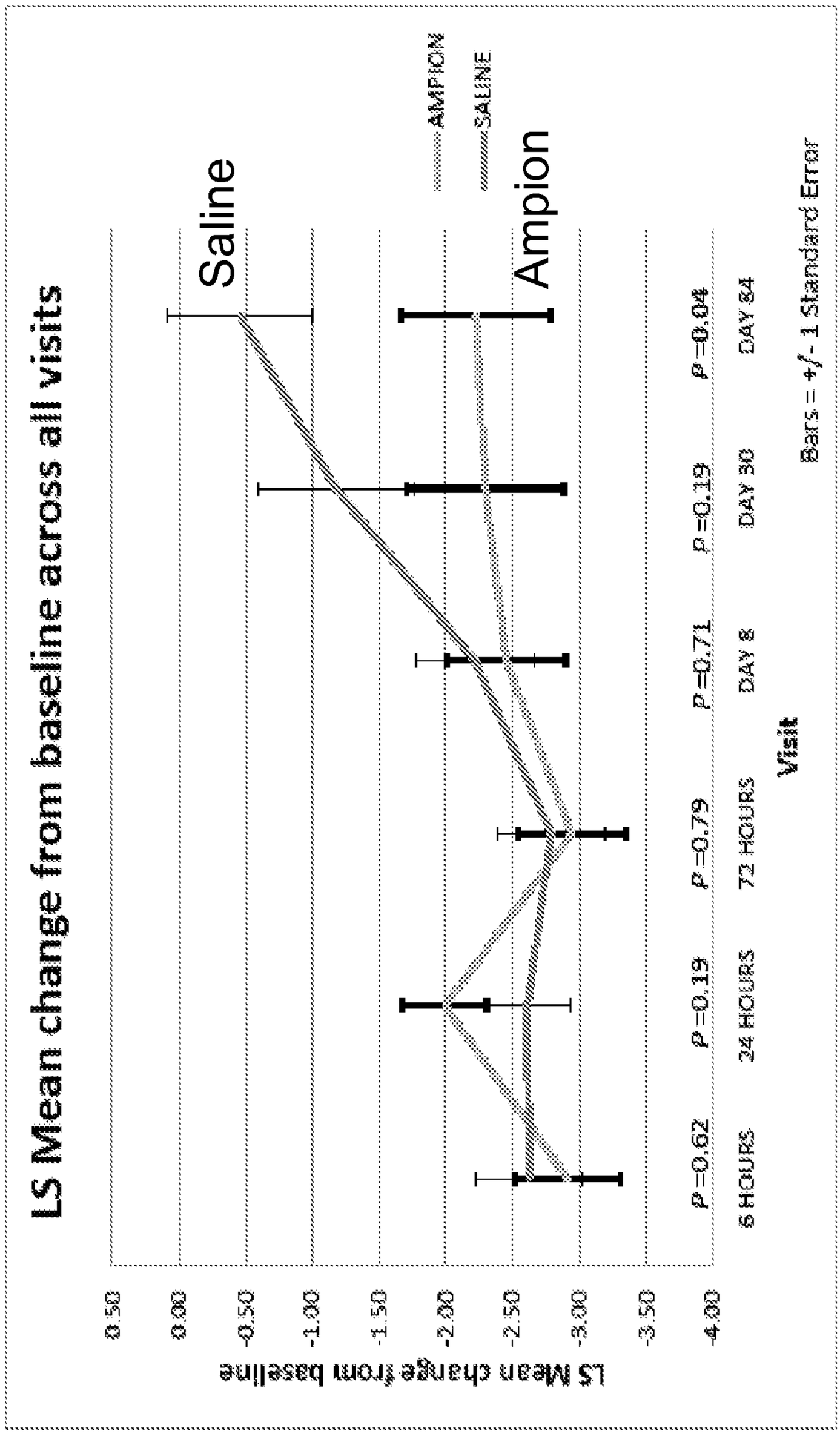
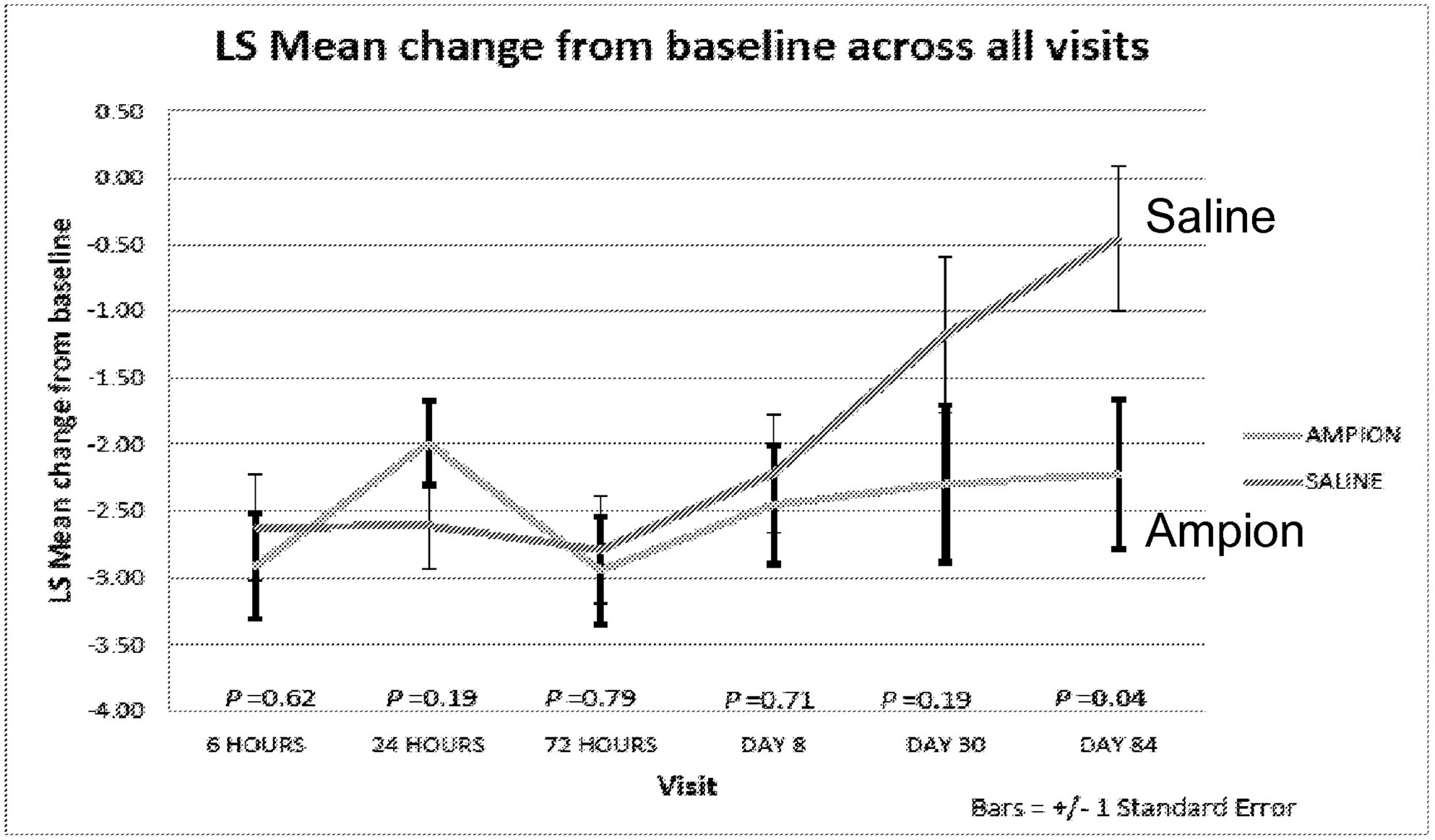

LS Mean change from baseline across all visits
Saline
Ampion
AMPION
SALINE
LS Mean change from baseline
0.50
0.00
-0.50
-1.00
-1.50
-2.00
-2.50
-3.00
-3.50
-4.00
P =0.62
P =0.19
P =0.79
P =0.71
P =0.19
P =0.04
6 HOURS
24 HOURS
72 HOURS
DAY 8
DAY 30
DAY 84
Visit
Bars = +/- 1 Standard Error

Figure 7

Figure 7


LS Mean change from baseline across all visits
LS Mean change from baseline
0.50
0.00
-0.50
-1.00
-1.50
-2.00
-2.50
-3.00
-3.50
-4.00
P =0.62
P =0.19
P =0.79
P =0.71
P =0.19
P =0.04
6 HOURS
24 HOURS
72 HOURS
DAY 8
DAY 30
DAY 84
Visit
AMPION
SALINE
Saline
Ampion
Bars = +/- 1 Standard Error