SYNTRA-5 DOWNREGULATES INFLAMMATORY SIGNALLING IN OBESE TYPE-2 DIABETES

MURINE MODEL IN VIVO

Syntra5™

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ABSTRACT

Background: Syntra-5, a dietary supplement, lowered blood glucose and improved several surrogate endpoints in a clinical study of diabetic patients. This study compared the activity of Syntra-5 to that of anti-diabetic drugs in an obese diabetic mouse model.

Methods: Syntra-5, metformin, Actos (pioglitazone hydrochloride), and Byetta (exenatide) were administered to BKS.Cg-m+/+Lepr^{db}/BomTac female mice. Untreated animals served as a control. Animals (8 per group) were fed either normal diet (ND) or high fat diet (HFD) +/- drugs for 8 weeks. Weight was measured weekly. We measured plasma levels of 40 biomarkers (chemokines, cytokines, endocrine, growth factors and metabolites) including insulin, glucose, advanced glycation end product (AGE), cholesterol and triglycerides. Pyruvate kinase activity, citrate, ADP and ATP concentrations and hexokinase II expression were determined in muscle tissue. Organ pathology was assessed visually and microscopically. Mean values of all 45 biomarkers were compared between treatment groups.

Results: Syntra-5's protection against organ damage was comparable with Byetta and better than Actos and metformin in the animals on ND. There was a slight decrease in the progression of morphological changes in the Syntra-5 group relative to the control group and other drug groups. The mean values of most plasma biomarkers were elevated in HFD relative to ND. Biomarker means varied significantly by treatment group and diet. On ND, Syntra-5 decreased levels of eotaxin, MCP-1, MCP-3, M-CSF, and increased IL-4 relative to untreated. Syntra-5 treatment decreased G-CSF, GM-CSF, and TGFβ relative to untreated in HFD animals. Pyruvate kinase and AGE increased, while insulin was decreased in animals treated with Syntra-5 relative to untreated on ND. Treatment group contrasts on biomarkers (MCP-3, IL-17, AGE, and insulin) also varied with diet.

Conclusions: Syntra-5 demonstrated superior anti-inflammatory activity relative to the commonly used anti-diabetic drugs on a background of genetic obesity. These results, taken together with the results of histological examination, support the contention that Syntra-5 may be an effective intervention for type 2 diabetes.

INTRODUCTION

Diabetes mellitus type 2 is a metabolic disorder that is characterized by high blood glucose in the context of insulin resistance and relative insulin deficiency. Insulin deficiency is associated with decreased rates of glycolysis, glycogenesis, lipogenesis, and protein synthesis. Under normal conditions, more than 80% of the energy produced by the body is derived from carbohydrate metabolism. When this metabolism is severely limited, as occurs in diabetes, the cell initiates oxidation of fat reserves for energy production and degrades proteins to amino acids which in turn are converted to glucose. If excessive fat metabolism occurs in conjunction with inadequate carbohydrate metabolism, one result is production of inadequate amounts of oxaloacetic acid with which to react with acetyl CoA from the fatty acid oxidation. An excess of acetyl CoA leads to a buildup of ketone bodies leading to ketosis and acidosis. Long term complications of the diabetic condition include arteriosclerosis and other cardiovascular problems, changes in the retina and the formation of cataracts, nervous system problems and kidney diseases. Among risk factors, a high fat diet is considered to be detrimental to people with diabetes (*1*). Dietary fat causes high blood sugar levels by preventing sugar from entering muscles and other cells, thus keeping sugar in the bloodstream and elevating it to high levels (*2*), (*3*). In addition, physical inactivity and obesity are two major risk factors for the development of type 2 diabetes (*4*).

Syntra-5™ is an herbal dietary supplement composed of vitamin C (ascorbic acid) 10 mg, biotin USP 100 mcg, chromium (chelate), hydroxycitrate 1600 mg, dicalcium phosphate, croscamellose sodium, stearic acid, silicon dioxide, magnesium stearate and hydroxypropyl methylcellulose. A recent double-blinded clinical study of diabetic patients demonstrated that

Syntra-5 lowered blood glucose levels, triglycerides, cholesterol, and blood pressure (Hampshire et al., unpublished data). To shed more light on Syntra-5's mode of action, we compared the activity of Syntra-5 to three commonly used anti-diabetic drugs: metformin, an anti-hyperglycemic agent that has been in use for nearly two decades (*5*) and two more recent drugs: Actos (pioglitazone hydrochloride) (*6*) and Byetta (exenatide) (*7*). The drugs were administered at doses comparable to those used in clinical practice in BKS.Cg-*m+/+Lepr*^{db}/BomTac female mice. These mice are homozygous for a point mutation in the gene for the leptin receptor LEP-R (*8*), a protein that in humans is encoded by the *LEPR* gene. The db/db mouse is a model of obesity, diabetes, and dyslipidemia (*9*) (*10*). LEP-R-deficient db/db mice develop disease similar to human type 2 diabetes mellitus, hypertension, and obesity with disrupted circadian blood pressure rhythm. The animals develop hyperglycemia at approximately 6 weeks age and frank immune dysfunction by 20 weeks (*11*).

Using this *in vivo* system, we selected a broad range of endpoints to evaluate mechanisms of action of Syntra-5. In addition to commonly tested metabolites (glucose, advanced glycation end product, insulin, cholesterol, triglycerides), we included pyruvate kinase, hexokinase II, and citrate as potentially relevant endpoints along with a large panel of cytokines, chemokines, endocrine markers and growth factors.

METHODS

Animals: The study was performed according to the guidelines for the care and use of laboratory animals at CPC LLC, San Antonio, Texas. BKS.Cg-*m+/+Lepr*^{db}/BomTac female mice that were 7 to 9 weeks old at shipment were sourced from Taconic Farms (Hudson, NY). The animals were housed until 10 to 12 weeks of age before start of treatment.

Animal diet and drug treatment: Animals were randomized into two groups of 40 mice each and fed *ad libitum* with Purina 5001 rodent diet (normal diet; ND) or BioServ High Fat Diet p.n. F1850 (BioServ, Frenchtown, NJ 08825) rodent diet paste (high fat diet; HF). Each diet group was further divided into five treatment groups (Actos, Byetta, metformin, Syntra-5, untreated) with 8 animals for each treatment group.

The recommended dosage of Syntra-5 is six capsules per day. Thus, if we refer to the maximum average 75th percentile body mass of white females at 82 kg the daily dosage is 6 x 1600 mg/82 kg = 117.12 mg/kg. The average body mass of BKS.Cg-m+/+Lepr^{db}/BomTac mice is ~20 g (0.02 kg) at 9 to 12 weeks of age. The average daily food consumption of an animal of this size is ~ 15 g / day. Thus, 117.12 mg x 0.02 kg = 2.3424 mg/kg/day of Syntra-5 in 15 g of food for a final concentration of 156.12 mg Syntra-5/kg of rodent diet. This diet was prepared by blending the appropriate quantities of Syntra-5 with HF paste. An equivalent drug formulation in ND was prepared by using water to soften the food granules reducing them to paste into which the various oral drugs were incorporated by mechanical mixing.

Metformin was formulated at 0.12% by weight in the two feed preparations. Actos typical daily dosage is 4 mg/day and the drug was formulated at 0.065 mg/kg in the two diets. Byetta was diluted in PBS to a final concentration of 12.2 ng/mL. The resulting solution was administered by

subcutaneous injection (100 µL) twice per day, once in the morning and once in the afternoon.

Tissue processing: At the termination of the experiment, at approximately 60 days, the animals were euthanized and exsanguinated. Whole peripheral blood was collected into BD Vacutainer CPT Cell Preparation Tubes (Beckton Dickinson, Franklin Lakes, NJ) and centrifuged at 1,500 x g at room temperature to isolate plasma according to supplier's protocol. Dissected organs (eyes, kidneys, muscle, pancreas, small and large intestine) were fixed in 10% phosphate buffered formalin (Fisher Scientific, Pittsburgh, PA). Tissue specimens were embedded in paraffin and stained with hematoxyllin and eosin. Muscle tissue was stained with rabbit monoclonal antibody to hexokinase II 64G5 (Cell Signaling, Danvers, MA) using MACH 2 Rabbit HRP-polymer (Biocare Medical, Concord, CA). Tissue images were generated in the Core Optical Imaging Facility, which is supported by University of Texas Health Sciences Center at San Antonio, NIH-NCI P30 CA54174 (CTRC), NIH-NIA P30 AG013319 (Nathan Shock Center) and NIH-NIA P01AG19316.

Evaluation of morphological changes: Morphological changes typical for diabetic pathology in the kidneys, eyes, muscular vasculature and pancreas were assessed by a pathologist who was blinded to the grouping. The changes were graded on a numeric scale from 0 being no lesion to 3 being a severe lesion. The pathology of the kidneys was evaluated and compared between animals based on the morphological changes in the glomeruli (thickening of the capillary basement membrane, diffuse mesangial sclerosis, nodular glomerulosclerosis and the thickening of Bowman capsule) and vasculature (arthero- and arteriolosclerosis). The ocular changes were assessed in relation to the presence of the thickening of the basement membranes in the in retinal vessels, retinal microhemorrhages, hemorrhagic

exudates in the outer plexiform layer, neovascularization of the internal limiting membrane and neovascularization of the vitreous humor. The reduction of the size and number of islets in the pancreas were compared between animals. The muscles were evaluated based on degree of hexokinase II immunohistochemical staining as well as on degree of thickening of the basement membrane in the small vessels. The intensity of hexokinase II staining was graded from 0 for no staining to 3 for uniform staining.

Bioassays: The plasma was analyzed by multiplexed immunoassay for the quantitative determination of 34 cytokines, chemokines and growth factors using Procarta cytokine profiling kits (Affymetrix, Fremont, CA). Advanced glycation end product (AGE) OxiSelect ELISA (#STA-317) was purchased from Cell BioLabs, San Diego, CA. Insulin ELISA kit (rat/mouse, # EZRMI-13K) was obtained from Linco Research, St. Charles, MO. Assay kits for glucose (#K613), pyruvate kinase (#K709), citrate (#K655), HDL and LDL/VLDL cholesterol (#K709), triglycerides (#K622), and Aposensor ADP/ATP ratio were obtained from BioVision (Mountain View, CA). For citrate assay, plasma was deproteinized using sample preparation kit from BioVision (#K808).

Statistical analysis: Continuously distributed outcomes were summarized with the mean and standard deviation. Relative values were graphically represented with a heat map. At each level of diet (normal, high fat), treatment groups were contrasted with regard to the mean using analysis of variance and for each biomarker, pairwise comparisons between treatment groups were corrected for multiple testing using the Tukey method. Treatment group contrasts with regard to weight were based on a repeated measures linear model with an autoregressive order 1 covariance assumption in terms of treatment, day, and the treatment by day interaction. All

statistical testing was two-sided with a nominal and experiment-wise significance level of 5% using SAS Version 9.2. R was used for graphics.

RESULTS

Body mass: Animal body weight (g) measured through the course of the study are summarized by treatment group in Figures 1 (normal diet) and 2 (high fat diet). In animals fed a normal diet body masses increased in a nearly linear fashion both in control animals and all drug treatment groups. In contrast, body masses of all mice maintained on high fat diet initially increased through first 3-4 weeks then started a gradual decline. The sacrifice time was determined based on animal appearance and performance, especially in the high fat diet mice. Mice in the control and Byetta treatment groups were sacrificed on Day 63. Animals treated with Syntra-5 were terminated on Day 56, while metformin and Actos-treated mice were sacrificed on Day 49 because of their largely compromised performance. There was one premature death in Byetta animals on normal diet and two deaths in Syntra-5 treated animals on high fat diet. There were no significant differences between the body mass curves in either diet.

Treatment group contrasts with Syntra-5 and Syntra-5 contrasts with untreated on mean weight at the end of study were not significant, regardless of diet [normal diet (Actos: 48.7±4.8, Byetta: 52.8±5.9, metformin: 49.3±3.9, Syntra-5: 47.3±8.0, untreated: 51.1±2.7), high fat diet (Actos: 52.0±2.3, Byetta: 44.6±2.0, metformin: 49.8±4.0, Syntra-5: 44.9±4.1, untreated: 42.1±5.9).

Necropsy: Control mice on normal diet appeared healthy with no visible wounds or scarring, good skin turgor and normal activity level. Large amounts of fat were noted in viscera. Liver, lungs, kidneys, pancreas, cardiac tissue and intestines were unremarkable. Rear leg muscles had some atrophy. Control mice on high fat diet appeared unhealthy, lethargic and nonreactive to environmental

stimuli. Ecchymosis was noted at the injection sites. Viscera were covered with abundant fat tissue. Pancreas was yellow in color and smaller than on normal diet. The intestines were yellowish and swollen.

Metformin-treated mice on normal diet were healthy with no visible wounds or scarring, had good skin turgor and normal activity level. There were no remarkable differences in comparison with respective control, except for mottled liver seen in most animals. Metformin-treated mice on high fat diet had large skin lesions and extensive hair loss. Ecchymosis was observed primarily at the injection sites. Skin had good turgor. The animals were lethargic and nonreactive to environmental stimuli. Viscera were yellowish and the liver had deposits of yellow adipose tissue. The pancreas was enlarged. There were diffuse tissue hemorrhages. The animals had poor blood clotting. Several animals had kidney/adrenal tumors.

All Actos-treated mice on normal diet appeared healthy but had decreased activity level. The kidneys looked healthy but were encapsulated in fat with leaky blood vessels. Liver was mottled in most animals. Large and small intestines were reddish in color. Mice treated with Actos plus high fat diet were clearly lethargic. A large amount of dense fat was seen within the upper peritoneal and retroperitoneal space. Kidneys were enlarged and encapsulated in adipose tissue. The pancreas was pale and enlarged. The liver had small deposits of yellow adipose tissue. The large and small intestines were yellow and swollen. The animals had some wasting of skeletal muscle and gross steatohepatits evidenced by diffuse yellow spotting. The animals did not bleed easily.

Mice treated with Byetta and maintained on normal diet were less active than the respective controls. Their viscera looked healthy but the kidneys were enlarged and the spleens were necrotic in many cases. The

pancreas was enlarged and sclerosed but had normal color and shape. The liver was mottled with fatty deposits. Other tissues were unremarkable. Byetta-treated mice on high fat diet looked unhealthy, lethargic, had ecchymosis at the injection sites, poor skin turgor and multiple head and neck lesions. The majority of animals showed signs of abdominal aortic aneurysm. The kidneys were enlarged and encapsulated in fat with leaky blood vessels. The pancreas was poorly perfused, pale in color, reduced in size and sclerosed. Both the liver and intestines were yellow. The livers had small deposits of fat and the intestines were swollen. The animals had hair loss and edema with some scabbing at injection sites indicative of poor wound healing.

The Syntra-5 treated mice on normal diet had no visible wounds or scarring and displayed good skin turgor. Their activity level was somewhat diminished in comparison with controls. The spleen, intestines, lungs and heart were unremarkable. The kidneys and pancreas were enlarged. The livers were mottled and necrotic with fatty deposits in most animals. Mice on high fat diet that were treated with Syntra-5 looked unhealthy and lethargic with visible ecchymosed tissue along the spine primarily at the injection sites, poor skin turgor and multiple lesions in the head and neck region. Visceral tissue was poorly oxygenated. Most organ appearance was similar to that of Syntra-5 plus normal diet. Some mice showed distinct signs of abdominal aortic aneurysm. Small and large intestines were yellow and swollen. Syntra-5 treated animals had hair loss and edema but not as extensive as the animals treated with Byetta.

Overall, based on appearance of the animals, drug efficacy followed the order: Syntra-5>Byetta>Actos>metformin.

Histological assessment of mouse tissues: There was an improvement in the expression of muscle hexokinase II in animals on Syntra-5 compared to the control animals, in particular on high fat diet (Figure 3A). The thickening of the basemen membrane as well as the degree of atherosclerotic changes in the

muscular and renal small vessels were less pronounced in animal group on Syntra-5 group than in control groups (metformin, Actos, Byetta, no treatment). We noted large differences between controls and Syntra-5 treated animals on high fat diet (Figure 3B). Similar results for mice on Syntra-5 compared to the controls were found by assessing proliferation of the glomerular mesangial matrix. The degree of glomerulosclerosis was not graded but was lower in the animals on Syntra-5 than in the animals in the other groups. The remaining morphological changes showed no differences between the animal groups.

Biomarker differences by treatment group and diet: Biomarker means varied significantly by treatment group and diet (Tables 1 and 2) and the mean values of most biomarkers were elevated in animals on high fat diet relative to normal diet.

Cytokines, chemokines, endocrine markers and growth factors: With the normal diet the mean eotaxin (ng/mL), MCP-1 (pg/mL) and MCP-3 (pg/mL) and M-CSF (pg/mL) were decreased after treatment with Syntra-5 relative to untreated [Eotaxin (Syntra-5: 2.7±0.5, Untreated: 5.3±1.9; p=0.005), MCP-1 (Syntra-5: 51.2±16.7, Untreated: 94.6±18.4; p<0.001), MCP-3 (Syntra-5: 224.1±98.3, Untreated: 526±72.6; p<0.001), M-CSF (Syntra-5: 5±3.1, Untreated: 9.3±2.4; p=0.04)] and with the high fat diet animals these mean values were decreased after with Syntra-5 but not significantly so.

The mean of IL-4 (pg/mL) was increased after treatment with Syntra-5 relative to untreated with both diets but this increase was significant only with the normal diet [normal diet (Syntra-5: 2.6±2.0, untreated: 0.8±0.5; p=0.04), high fat diet (Syntra-5: 2.0±2.2, untreated: 0.7±0.6; p=0.22)].

The mean of the growth factors G-CSF, GM-CSF, and TGF were significantly decreased in animals treated with Syntra-5 relative to untreated in the high fat diet [normal diet G-CSF (Syntra-5: 73.2±87.2, Untreated: 44.3±40.1; p=1.0), GM-CSF (Syntra-5: 182.4±256.6, Untreated: 226.6±535.2; p=0.98), TGFβ (Syntra-5: 54.8±25.6, Untreated: 83.6±74.9; p=0.93), High Fat Diet G-CSF (Syntra-5:

56.2±74, Untreated: 735.8±769.6; p=0.02), GM-CSF (Syntra-5: 81.9±129.7, Untreated: 457.8±433.1; p=0.03), TGFβ (Syntra-5: 22.5±34.3, Untreated: 74.9±72.1; p=0.005)].

Metabolites: The mean pyruvate kinase (mU/mL) was increased in animals treated with Syntra-5 relative to untreated in both diets, but significantly so with the normal diet [Normal diet (Syntra-5: 2.1±0.9, Untreated: 0.7±0.9; p=0.02) High Fat Diet (Syntra-5: 2.0±0.8, Untreated: 1.6±0.8; p=0.94)].

The mean advanced glycation end product (mg/dL) (Figures 4 and 5) was increased after treatment with Syntra-5 relative to untreated with both diets and the increase was significant only with the normal diet [Normal diet (Syntra-5: 5.4±2.1, Untreated: 1.6±0.6; p<0.001), High Fat Diet (Syntra-5: 2.4±1.8, Untreated: 1.7±0.8; p=0.90)].

The mean insulin (ng/mL) (Figures 6 and 7) was significantly decreased after treatment with Syntra-5 relative to untreated with the normal diet and non-significantly increased with the high fat diet [Normal diet (Syntra-5: 4.4±4.9, Untreated: 16.8±6.0; p<0.001) High Fat Diet (Syntra-5: 3.5±1.3, Untreated: 3.0±4.6; p=0.55)].

The mean citrate (mM) (Figures 8 and 9) was increased after treatment with Syntra-5 with both diets, significantly so with the high fat diet [normal diet (Syntra-5: 0.4±0.24, Untreated: 0.21±0.19; p=0.09), high fat diet (Syntra-5: 0.63±0.16, Untreated: 0.22±0.27; p=0.007)].

Treatment group contrasts: Treatment group contrasts on biomarker means varied with diet and biomarker. With the normal diet, the mean MCP-3 (pg/mL) was significantly increased after treatment with Actos, Byetta and metformin relative to Syntra-5 [Actos: 460.7±189.9 (p<0.001), Byetta: 476.0±99.9 (p<0.001), metformin: 502.7±175.8 (p<0.001), Syntra-5: 224.1±98.3]; the corresponding contrasts were not significant with the high fat diet [Actos: 757.8±193.2 (p=0.81),

Byetta: 860.5±177.5 (p=0.57), metformin: 457.0±287.7 (p=0.94), Syntra-5: 455.7±433.1].

With the high fat diet, the mean IL-17 (pg/mL) was decreased after treatment with Syntra-5 relative to Actos, Byetta and metformin, significantly so relative to Byetta [Normal diet, Actos: 66.2±41.5 (p=0.51), Byetta: 133.0±53.5 (p=0.81), metformin: 144.5±127.7 (p=0.91), Syntra-5: 104.6±54.8, High fat diet, Actos: 439.0±230.0 (p=0.09), Byetta: 953.4±660.5 (p<0.001), metformin: 377.2±181.2 (p=0.18), Syntra-5: 196.4±159.8].

With the normal diet, the mean advanced glycation end product (mg/dL) was significantly decreased after treatment with Actos and metformin relative to Syntra-5 [Actos: 1.6±0.3 (p<0.001), Byetta: 4.4±1.8 (p=0.79), metformin: 1.9±0.7 (p<0.001), Syntra-5: 5.4±2.1]; the corresponding contrasts were not significant with the high fat diet [Actos: 2.5±1.9 (p=1.0), Byetta: 2.8±2.5 (p=1.0), metformin: 1.3±0.2 (p=0.64), Syntra-5: 2.4±1.8].

With the normal diet, the mean insulin (ng/mL) was significantly increased after treatment with Actos, Byetta and metformin relative to Syntra-5 [Actos: 8.8±4.5 (p=0.04), Byetta: 11.6±4.9 (p=0.004), Metformin: 20.7±2.5 (p<0.001), Syntra-5: 4.4±4.9]; the corresponding contrasts were not significant with the high fat diet [Actos: 4.4±3.6 (p=1.0), Byetta: 1.4±0.4 (p=0.73), metformin: 4.6±4.1 (p=1.0), Syntra-5: 3.5±1.3].

Bivariate clustering by treatment group and biomarker was carried out for each diet and summarized with heat maps (Figures 10 and 11); in these figures high levels of a biomarker are represented in blue, low levels in green and missing values are indicated with white. Comparison of Figure 10 (normal diet) with Figure 11 (high fat diet) shows variation with both biomarker and treatment. For example with normal diet (Figure 10), triglycerides, HDL, advanced glycation end product form a separate cluster but these are not clustered with the high fat diet (Figure 11). In the normal diet (Figure 10) but not in the high fat diet (Figure 11) Syntra-5 tends to cluster near untreated.

DISCUSSION

The study illustrated the biochemical activity of Syntra-5 relative to the commonly used anti-diabetic drugs against a background of genetic obesity. Analysis of the patterns of cytokine and other biomarkers regulation provided insights into how Syntra-5 exerts its salutary effects in diabetes by significantly regulating expression of a broad range of surrogate markers of diabetic severity.

Using animal performance status as the endpoint we concluded that Syntra-5 and Byetta were better tolerated than metformin and Actos. Increased mortality in the high fat diet of Syntra-5 treated animals did not come as a surprise. Given high concentration of hydroxycitrate in Syntra-5 preparation, feeding the animals with lipids as a primary food source resulted in shutting down the tricarboxylic acid cycle and effectively starving of the animals. In fact, this demonstrated the efficacy of Syntra-5 in the experimental diabetes model.

There was a slight but visible reduction in the progression of morphological changes in the animal on Syntra-5 compared to the control group. The most pronounced morphological differences were observed in the proliferation of vascular basement membranes and the proliferation of the glomerular mesangial matrix. These are structures where changes develop earlier compared to the other structures examined. Observed pathologies including diabetic glomerulosclerosis (*12*) and vascular basement membrane thickening that is viewed as the main structural alteration of small blood vessels in diabetes (*13*), responded favorably to treatment with Syntra-5. Our findings are consistent with improvement of similar diabetes symptoms by experimental drugs in animal model of experimental diabetes (*14*). The absence of significant differences among the groups on other evaluated lesions can be explained as being due to the relatively short duration of the experiment which was not long enough for injuries to develop.

Hexokinase II is a key glycolytic enzyme and an important biomarker of diabetes. Hexokinase II downregulation in diabetic muscle results from decreased synthesis coupled to increased degradation relative to the normal tissue in rodents (*14*) and humans (*15*). Evident upregulation of hexokinase II by Syntra-5 treatment was similar to the effect of Actos in a recent study using murine model of human type II diabetes (*16*).

We noted several surprising findings regarding Syntra-5 and other drug effects on select metabolites, in particular glucose, triglycerides, HDL and LDL plus VLDL cholesterol fractions, and ADP and ATP levels, which were not significantly affected by any treatment on normal or high fat diet. These results are likely an outcome of the in vivo model system, which nevertheless shares only some characteristics with human type 2 diabetes (*17*).

Significant inter-group differences in expression levels of several markers were dependent on the diet. Advanced glycation end product (AGE), which is exemplified by glycated hemoglobin (hemoglobin A1c), is formed in a non-enzymatic pathway by exposure of plasma proteins to high levels of glucose (*18*). Protein glycation is associated with cardiovascular disease, nephropathy and retinopathy. Although Syntra-5 treatment in animals on normal diet was associated with increased advanced glycation end product concentrations, a similar effect was also observed for Byetta. Since the levels of advanced glycation end product are proportional not only to concentration of plasma glucose but also to the half-life of target proteins and are genetically determined (*19*), it is conceivable that both Syntra-5 and Byetta might have affected turnover of glycated proteins in this in vivo model.

We feel that metrics, such as advanced glycation end product level, should be considered more in the frame of being disease symptoms rather than being elevated to the status of disease and/or mechanisms of disease. These markers are useful but they should not be seen as levers that can be grasped to

alter physiology - they are more like pressure gauges on a steam engine. Blood sugar level for instance has been twisted into this status. Many think that by 'controlling' i.e. lowering blood sugar, that the disease is controlled. Yet even with perfect glucose control there are still problems. Drugs that are designed to control blood sugar for instance have many liabilities such as hypoglycemia. Thus the emphasis on controlling blood sugar may be misplaced. It is much more important to look at the causes of diabetes, i.e. the immune system. High blood sugar should be seen for what it is - a symptom of disease, not a disease in and of itself. We are not saying, however, that high blood sugar is not a problem, because it is, but it is not the whole problem. Therefore, the cytokine results are extremely important in supporting contentions about the effect of the drug on the disease, not just the symptoms. The insights gained may also guide decisions about composition of the drug.

Insulin, which regulates the level of blood glucose, is still produced in the pancreas by the Langerhans isles in type 2 diabetes, however the response of cells throughout the body is abnormal. By reducing the concentration of glucose in the blood, insulin is thought to reduce the long-term complications of diabetes, including damage to the blood vessels, eyes, kidneys and nerves (*20*). In this study, Syntra-5 induced significant hypoinsulinemia on normal diet. This can be viewed as a beneficial effect of Syntra-5 considering that the ob/ob mice with a deficiency in the leptin pathway have elevated insulin levels (*21*), (*22*).

Pyruvate kinase, which regulates the rate-limiting final step of glycolysis, catalyzes conversion of phosphoenolpyruvate to pyruvate, which enters the citric acid cycle after conversion to acetyl-CoA and reaction with oxaloacetate to produce citric acid. In diabetes the rate of glycolysis is decreased and some of that effect is attributable to pyruvate kinase deficiency. The assayable activity of skeletal pyruvate kinase does not always reflect enzyme concentration (*23*).

Pyruvate kinase is reversibly inhibited by phosphorylation or ATP alone. In addition low blood glucose induces phosphorylation. Syntra-5 elevated pyruvate kinase activity on normal diet while other drugs had no significant effects. Increased activity of pyruvate kinase upon treatment with Syntra-5 is unlikely to be due to reduced glucose or ATP levels, as these markers were not significantly different from the control. On the other hand, long term exposure to hydroxycitrate, one of the main ingredients of Syntra-5, may lead to upregulation of pyruvate kinase expression. Examples of such drug-induced upregulation were reported before (*24*).

Citrate is an intermediate in the tricarboxylic acid cycle, the main energy source of the cell. After the pyruvate dehydrogenase complex forms acetyl CoA from pyruvate and cofactors (thiamine pyrophosphate, lipoamide, FAD, NAD+ and CoA), citrate synthase catalyzes the condensation of oxaloacetate with acetyl CoA to form citrate. Citrate was significantly increased by Syntra-5 only on high fat diet, consistent with inefficient tricarboxylic acid cycle operation and utilization of citrate.

In the past few years diabetes is being increasingly recognized as an inflammatory disease in which obesity plays a key role in the metabolic syndrome related to insulin resistance and heightened risk for the development of type 2 diabetes. Obesity is associated with low-grade chronic inflammation associated with the adipose tissue. In turn, the activated inflammatory cascade cross-talks with insulin signaling pathways through induction of cytokines, chemokines and growth factors (*25*). Circulating biomarkers of the inflammatory pathway, ion cascade, endothelial dysfunction, and procoagulant imbalance may be associated with development of both type 1 and type 2 diabetes (*21*). The comorbidities of diabetes, such as obesity, insulin resistance, hyperglycemia, hypertension and dyslipidemia, further aggravate the nephropathy, retinopathy and cardiovascular disease while antihyperglycemic therapies relieve these symptoms (*26*), (*27*).

Remarkably, Syntra-5 significantly downregulated a range of pro-inflammatory chemokines, cytokines, and growth factors. The roles of eotaxin (*28*), MCP-1 (*29-30*), MCP-3 (31) and M-CSF (*32-33*) in maintenance of the inflammatory state are well documented. IL-17 is of particular importance, as the IL-23/IL-17 pathway is a key player in chronic inflammation (34), (35). GM-CSF is a major regulatory factor that controls the functions of granulocyte and macrophage lineage populations at all stages of maturation and plays a key role in inflammatory and autoimmune diseases (*36*). TGFβ acting in concert with inflammatory cytokines supports de novo differentiation of IL-17-producing T cells (*37*). In addition, Syntra-5 suppressed circulating levels of a number of important pro-inflammatory markers including the chemokine KC that is homologous to human IL-8 (*38*), IL-6 (*39*), IFNγ (*40*) and VEGF (*41*) but also elevated levels of anti-inflammatory cytokine IL-4 (*42-43*), yet another therapeutically significant target. Mice expressing IL-4 in the pancreas are protected from diabetes (*44*), and the administration of recombinant IL-4 prevents diabetes in diabetic mice (*45*).

Macrophage activity is intimately connected to cytokines and pancreatic islets β-cell death in type 2 diabetes. The islets from patients with Type 2 diabetes are infiltrated by immune cells that produce inflammatory factors (IL-6, KC/IL-8, G-CSF, MIP-1α and MCP-1). Increased islet-associated macrophages are observed in human type 2 diabetic patients and in most animal models of diabetes, and in islets of mice on high fat diet even before the onset of diabetes (*33*). Syntra-5, by virtue of downregulating relevant inflammatory molecules and upregulating anti-inflammatory molecules, may break this vicious cycle and slow down the process of islets destruction.

Since one of the characteristics of diabetes is derangement of the immune system, and because the markers measured are all immune system messages and effectors, differences from control are indicative of drug effects. In

normal immune function, the inflammatory markers peak first in response to some stimulus, for instance in the case of infection. Then the anti-inflammatory markers step in at a later time to limit the damage caused by the pro-inflammatory markers. In this way the immune system mobilizes to first attack an invader and then calls a cease fire to keep collateral damage down. In autoimmune diseases, such as diabetes, the coordination of the pro- and anti-inflammatory mechanisms seems to be broken. For instance both can be elevated simultaneously. This kind of discoordination is common in many immune mediated diseases.

Mechanistically, the functions of cytokines, chemokines and growth factors are reasonably well understood. Many pro-inflammatory factors are viewed as novel therapeutic targets for the treatment of chronic inflammatory diseases, for example IL-17 or GM-CSF (*34-36*). Syntra-5 has been shown to downregulate these markers. Given that it is a defined mixture, knowing how the complete product and each of its components affect the immune system is of vital importance. This knowledge should provide a mechanistic basis for how the product works. The immune system and its effectors are the primary mediators of cellular and organ damage in diabetes. Thus connecting the effect of the drug to the immune system will allow us to say with a great deal of confidence how the drug causes its beneficial effect. These results, when considered in light of the results of histological examination, support the contention that Syntra-5 may be an effective intervention for type-2 diabetes.

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ABBREVIATIONS

AGE	advanced glycation end product
GM-CSF	granulocyte macrophage-colony stimulating factor
HDL	high-density lipoprotein
HFD	high fat diet
IL	interleukin
LDL	low-density lipoprotein
MCP-1	monocyte chemoattractant protein 1
ND	normal diet
VLDL	very low-density lipoprotein

FIGURE LEGENDS

Figure 1. Weight (g) versus day by treatment group (normal diet).

Figure 2. Weight (g) versus day by treatment group (high fat diet).

Figure 3A. An example of muscle hexokinase II in an animal treated with Syntra-5 (left) versus an untreated animal (right) on high fat diet.

Figure 3B. An example of renal tissue in an animal treated with Syntra-5 (left) versus an untreated animal (right) on high fat diet.

Figure 4. Advanced glycation end product (mg/dL) by treatment group with the normal diet; significant contrasts ($p<0.05$) with Syntra-5 (*), significant contrast with untreated (†).

Figure 5. Advanced glycation end product (mg/dL) by treatment group with the high fat diet; significant contrasts ($p<0.05$) with Syntra-5 (*), significant contrast with untreated (†).

Figure 6. Insulin (mg/dL) by treatment group with the normal diet; significant contrasts ($p<0.05$) with Syntra-5 (*), significant contrast with untreated (†).

Figure 7. Insulin (mg/dL) by treatment group with the high fat diet; significant contrasts ($p<0.05$) with Syntra-5 (*), significant contrast with untreated (†).

Figure 8. Citrate (mM) by treatment group with the normal diet; significant contrasts ($p<0.05$) with Syntra-5 (*), significant contrast with untreated (†).

Figure 9. Citrate (mM) by treatment group with the high fat diet; significant contrasts ($p<0.05$) with Syntra-5 (*), significant contrast with untreated (†).

Figure 10. Heat map (normal diet). Advanced glycation end product is shown as AGE.

Figure 11. Heat map (high fat diet). Advanced glycation end product is shown as AGE.

Syntra-5 Mice Study (6 December 2010)

Table 1. Biomarker Levels by Treatment (Normal Diet Only)

		Treatment				
		Actos	Byetta	Metformin	Syntra-5	Untreated
Chemokine	BTC (pg/mL)	12.2±28.2	14.4±26.6	54.2±122.8	29.3±39.3	38.1±96.2
	Eotaxin (ng/mL)	3.9±1	3.1±0.5	3.3±0.9	**2.7±0.5**[†]	5.3±1.9
	KC (pg/mL)	64.7±33.5	52.6±27.6	102.6±117.7	89.7±60.6	145.1±175.1
	LIF (pg/mL)	22.7±23.2	36.1±6.9	29.5±22.3	33.1±18.4	19.6±6.7
	LIX (ng/mL)	8.6±1.7	9.9±1.8	8.3±3.5	7.8±0.9	10.1±2.1
	MCP-1 (pg/mL)	65.3±16	**76.3±13.9**[*]	67.6±19.9	**51.2±16.7**[†]	94.6±18.4
	MCP-3 (pg/mL)	**460.7±189.9**[*]	**476±99.9**[*]	**502.7±175.8**[*]	**224.1±98.3**[†]	526±72.6
	MCSF (pg/mL)	5.9±1.8	6.7±0.8	**8.9±2.5**[*]	**5±3.1**[†]	9.3±2.4
	MIP-1 alpha (pg/mL)	22.9±20.7	28.4±7.4	33.3±25.5	27±16.8	16.4±5.9
	MIP-2 (pg/mL)	52.7±15.5	61.3±3.7	58.8±15.2	54.7±19.3	54.3±6.8
	RANTES (pg/mL)	25.9±13.3	42.1±18	41.2±18.4	29.9±6.7	28±21.4
	sRANKL (pg/mL)	31.6±27.4	23.3±4.7	33.5±12.3	23.2±7.9	26.5±6.3
Cytokine	IL-17 (pg/mL)	66.2±41.5	133±53.5	144.5±127.7	104.6±54.8	118.9±69.8
	IFN-Gamma (pg/mL)	56.2±95.7	30±71.1	134±349.9	116.4±202.4	188±493.8
	IL-1 beta (pg/mL)	95.7±31.6	106±12.8	129±47.9	100.7±15.9	119.1±32.3
	IL-1 alpha (pg/mL)	28.8±5.3	31±4.9	32.9±8	27.3±4.6	34.6±6.9
	IL-2 (pg/mL)	0±0	0±0	0±0	0±0	27.4±77.6
	IL-3 (pg/mL)	3.8±8.5	8.1±16.1	12.9±31.5	8.9±11.2	13.2±33.6
	IL-4 (pg/mL)	1.7±2.2	2.4±0.8	1.1±1.3	**2.6±2**[†]	0.8±0.5
	IL-5 (pg/mL)	8.5±5.1	9.4±3.7	10±5.8	8.3±4.9	5.9±2.2
	IL-6 (pg/mL)	9.8±8.1	11.4±2.5	30.8±59.4	13.7±9.9	17±12.9
	IL-9 (pg/mL)	31.2±38.4	7.1±9.3	24±23.6	1.5±4.3	16.9±7.7
	IL-10 (pg/mL)	0±0	0±0	0±0	0±0	0±0
	IL-12 p40 (pg/mL)	75.6±26.2	154.8±17.9	129.2±34.9	127.7±68.8	131±51.1

[*]Treatment is significantly different[12] from Syntra-5.
[†]Syntra-5 is significantly different[12] from Untreated.
[1]Based on a linear model of the logarithm of the values.
[2]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 1. Biomarker Levels by Treatment (Normal Diet Only)

		Treatment				
		Actos	Byetta	Metformin	Syntra-5	Untreated
Cytokine	IL-12 p70 (pg/mL)	44.4±33	59.9±9	51.7±23.3	52.4±27.9	40.9±25.4
	IL-13 (pg/mL)	3.9±4.7	7.3±6.2	7.5±9.8	8.7±5.5	9.3±18.1
	IL-23 (pg/mL)	7.8±11.1	13±10.2	26.6±30.6	27.5±25.3	18.8±12.1
	IP-10 (pg/mL)	616.3±486.2	572.1±445	1074.9±2178.9	1125.1±1506.2	1161.6±2405.2
Endocrine	Adiponectin (ng/mL)	245.4±77.9	211.5±43.6	199.1±34.7	190.4±29	265±39.4
	Leptin (ng/mL)	32.1±15.4	28.1±4.4	36.5±30.4	30.3±14.8	43.7±10.8
Growth Factor	G-CSF (pg/mL)	29.5±25	20±7.8	225.2±530.8	73.2±87.2	44.3±40.1
	GM-CSF (pg/mL)	63.1±80.3	94.8±144.4	160.6±350.5	182.4±256.6	226.6±535.2
	TGF beta (pg/mL)	29±21.4	34.4±10	41.8±40.7	54.8±25.6	83.6±74.9
	TNF alpha (pg/mL)	7±6	8±3.8	11±13.5	13.3±11.7	12±2.4
	VEGF (pg/mL)	8.8±5.6	7.8±1.9	8.9±4.5	7±2.6	10.5±3
Metabolites	Glucose (mg/dL)	153.7±80	126.1±15	131.7±20.2	128.6±19.8	111.7±12
	Triglycerides (mM)	0.02±0.01	0.03±0.01	0.02±0.01	0.02±0.02	0.01±0.01
	HDL (μg/μL)	0.49±0.07	0.53±0.12	0.41±0.11	0.51±0.17	0.41±0.1
	LDL & VLDL (μg/μL)	0.13±0.03	0.14±0.04	0.15±0.02	0.11±0.05	0.12±0.03
	ATP (nM)	527.6±448	704.5±487.2	165.1±81.4	533.7±495.8	3885.4±5860.4
	ADP (μM)	1.8±1.1	1.9±1	0.8±0.3	1.7±1.1	5.1±6.6
	Pyruvate Kinase (mU/mL extract)	1.8±1	1.2±0.7	1.7±0.8	2.1±0.9	0.7±0.9
	Citrate (mM)	0.47±0.31	0.56±0.17	0.79±0.21	0.4±0.24	0.21±0.19
	Advanced Glycation End Product (mg/dL)	**1.6±0.3**[*]	4.4±1.8	**1.9±0.7**[*]	**5.4±2.1**[†]	1.6±0.6
	Insulin (ng/mL)	**8.8±4.5**[*]	**11.6±4.9**[*]	**20.7±2.5**[*]	**4.4±4.9**[†]	16.8±6

[*]Treatment is significantly different[12] from Syntra-5.
[†]Syntra-5 is significantly different[12] from Untreated.
[1]Based on a linear model of the logarithm of the values.
[2]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 2. Biomarker Levels by Treatment (High Fat Diet Only)

		Treatment				
		Actos	Byetta	Metformin	Syntra-5	Untreated
Chemokine	BTC (pg/mL)	141.3±125.3	67.4±68.6	77.2±68.4	23.2±47.4	153.3±122.3
	Eotaxin (ng/mL)	3.8±1	2.9±0.5	3.5±1.6	2.3±0.6	3.8±2
	KC (pg/mL)	237.2±217.3	135.6±121.5	210±159.2	64.2±40.2	254.7±291.1
	LIF (pg/mL)	30.8±15.2	30.2±10.1	52±40.4	24.9±11.4	21.2±13.3
	LIX (ng/mL)	8.2±3.3	6±2	4.4±1.9	6.6±2.8	8.4±6.9
	MCP-1 (pg/mL)	159±52	167.3±50.3	157±91.8	185.2±166.8	230.2±142.6
	MCP-3 (pg/mL)	757.8±193.2	860.5±177.5	457±287.7	455.7±433.1	1216.2±1456.3
	MCSF (pg/mL)	7.4±3	10.6±16.5	5.5±2.5	6.4±1.5	9.8±4.1
	MIP-1 alpha (pg/mL)	18.5±11.4	19.6±12.7	34.4±31.6	196±433.2	7.3±6
	MIP-2 (pg/mL)	65.1±17.3	66.9±26	62.1±11.4	49.7±9.8	58.5±15.2
	RANTES (pg/mL)	26.1±11.9	21.3±16.8	20.5±8.2	11.8±9.9	42.3±56.8
	sRANKL (pg/mL)	30.1±24.9	26.5±9	18±9	29.5±30.7	37.6±36.9
Cytokine	IL-17 (pg/mL)	439±230	**953.2±660.5**[*]	377.2±181.2	196.4±159.8	502±723.3
	IFN-Gamma (pg/mL)	577.2±593.8	201.4±259	325.8±378.7	82.1±183.5	426.3±511.5
	IL-1 beta (pg/mL)	122.6±20	124.1±55.6	130.2±41.2	96.9±56.7	150.9±154.7
	IL-1 alpha (pg/mL)	43.2±20.3	53.1±48.4	29.6±8.3	28.6±8.4	32.6±12
	IL-2 (pg/mL)	40.2±113.8	0±0	24.8±70.3	0±0	49.7±140.7
	IL-3 (pg/mL)	52±46.5	22.8±19.6	22.7±19.4	12.6±25.8	43.2±51.1
	IL-4 (pg/mL)	1.6±1.6	1.7±1	3.2±3.9	2±2.2	0.7±0.6
	IL-5 (pg/mL)	9.7±5.6	11.9±8.3	14.4±6.9	14±7.6	14.7±22.5
	IL-6 (pg/mL)	40.5±17.1	82.6±69.4	45±22.4	27.4±28.8	117.8±124.5
	IL-9 (pg/mL)	23.1±25.3	8.8±11.4	2.4±5.9	14.6±26.9	18±22
	IL-10 (pg/mL)	0±0	0±0	0±0	0±0	3.3±9.34
	IL-12 p40 (pg/mL)	135.3±33.9	157±99.4	179.8±87.9	103.4±75.4	278±415.6

[*]Treatment is significantly different[12] from Syntra-5.
[†]Syntra-5 is significantly different[12] from Untreated.
[1]Based on a linear model of the logarithm of the values.
[2]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 2. Biomarker Levels by Treatment (High Fat Diet Only)

		Treatment				
		Actos	Byetta	Metformin	Syntra-5	Untreated
Cytokine	IL-12 p70 (pg/mL)	61±24	95.9±97.6	86.8±64.1	47.5±31.7	138±255.1
	IL-13 (pg/mL)	22.4±13.7	16.6±13.1	20.3±12.4	8.9±13.2	26.5±44.1
	IL-23 (pg/mL)	5.2±6.6	22.1±36.1	15.7±15.6	15.9±15.6	8.9±16.6
	IP-10 (pg/mL)	3212±3381.7	1313.5±1279.1	1862.4±1204.7	486.5±466.1	2362.3±1922.5
Endocrine	Adiponectin (ng/mL)	228.4±53.8	236.1±44.7	193±68.3	210.6±115.1	302±176.6
	Leptin (ng/mL)	20.8±10.1	9.7±2.9	29.8±31.7	18±9.9	15.9±12.3
Growth Factor	G-CSF (pg/mL)	252.9±189.7	**1378.1±1507.8**[*]	135.2±91.9	**56.2±74**[†]	735.8±796.6
	GM-CSF (pg/mL)	**527.1±449.5**[*]	266.4±267.2	425.1±424.9	**81.9±129.7**[†]	457.8±433.1
	TGF beta (pg/mL)	**56.2±30.8**[*]	45.8±29.9	33±24	**22.5±34.3**[†]	74.9±72.1
	TNF alpha (pg/mL)	10.4±5.6	15.3±8.8	28.5±31.6	4.2±6.4	15±7.9
	VEGF (pg/mL)	6.6±3.2	9.1±3	9.2±5.6	6.2±4.8	9.9±2.5
Metabolites	Glucose (mg/dL)	291.3±202.3	412.4±195.5	343.9±226.7	224±42.4	189.7±65.7
	Triglycerides (mM)	0.02±0.01	0.02±0.01	0.02±0.01	0.01±0.01	0.02±0.01
	HDL (μg/μL)	0.51±0.18	0.43±0.18	0.46±0.09	0.57±0.17	0.43±0.08
	LDL & VLDL (μg/μL)	0.15±0.04	0.14±0.05	0.24±0.1	0.19±0.08	0.22±0.12
	ATP (nM)	397.8±467.4	269.1±203.1	146.4±111.3	520.5±421.1	270.1±144.6
	ADP (μM)	1.5±1.1	1.4±0.7	0.9±0.4	2.2±1.3	1.6±0.7
	Pyruvate Kinase (mU/mL extract)	2.1±1.3	2.1±1.7	3.2±3.1	2±0.8	1.6±0.8
	Citrate (mM)	0.48±0.31	0.63±0.32	0.62±0.34	**0.63±0.16**[†]	0.22±0.27
	Advanced Glycation End Product (mg/dL)	2.5±1.9	2.8±2.5	1.3±0.2	2.4±1.8	1.7±0.8
	Insulin (ng/mL)	4.4±3.6	1.4±0.4	4.6±4.1	3.5±1.3	3±4.6

[*]Treatment is significantly different[12] from Syntra-5.
[†]Syntra-5 is significantly different[12] from Untreated.
[1]Based on a linear model of the logarithm of the values.
[2]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 3. Chemokine Levels by Treatment (Normal Diet Only)

	Treatment					
	Actos	Byetta	Metformin	Syntra-5	Untreated	P-Value
BTC (pg/mL)	12.2±28.2	14.4±26.6	54.2±122.8	29.3±39.3	38.1±96.2	0.08[1]
P-Value[23] for pairwise comparison:						
versus Actos		1	0.98	0.95	1	
versus Byetta			0.97	0.94	1	
versus Metformin				1	0.98	
versus Syntra					0.94	
Eotaxin (ng/mL)	3.9±1	3.1±0.5	3.3±0.9	2.7±0.5	5.3±1.9	0.27[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.56	0.76	0.09	0.44	
versus Byetta			1	0.76	0.045	
versus Metformin				0.54	0.08	
versus Syntra					0.005	
KC (pg/mL)	64.7±33.5	52.6±27.6	102.6±117.7	89.7±60.6	145.1±175.1	0.8[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.96	0.95	0.93	0.5	
versus Byetta			0.65	0.61	0.2	
versus Metformin				1	0.9	
versus Syntra					0.92	

[1]F Test
[2]Based on a linear model of the logarithm of the values.
[3]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 3. Chemokine Levels by Treatment (Normal Diet Only)

	Treatment					
	Actos	Byetta	Metformin	Syntra-5	Untreated	P-Value
LIF (pg/mL)	22.7±23.2	36.1±6.9	29.5±22.3	33.1±18.4	19.6±6.7	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.3	0.67	0.68	0.96	
versus Byetta			0.97	0.95	0.66	
versus Metformin				1	0.95	
versus Syntra					0.96	
LIX (ng/mL)	8.6±1.7	9.9±1.8	8.3±3.5	7.8±0.9	10.1±2.1	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.95	0.88	1	0.92	
versus Byetta			0.48	0.8	1	
versus Metformin				0.98	0.41	
versus Syntra					0.75	
MCP-1 (pg/mL)	65.3±16	76.3±13.9	67.6±19.9	51.2±16.7	94.6±18.4	<0.001[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.73	1	0.33	0.047	
versus Byetta			0.81	0.03	0.53	
versus Metformin				0.26	0.07	
versus Syntra					<0.001	

[1]F Test
[2]Based on a linear model of the logarithm of the values.
[3]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 3. Chemokine Levels by Treatment (Normal Diet Only)

	Treatment					
	Actos	Byetta	Metformin	Syntra-5	Untreated	P-Value
MCP-3 (pg/mL)	460.7±189.9	476±99.9	502.7±175.8	224.1±98.3	526±72.6	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.98	0.95	<0.001	0.73	
versus Byetta			1	<0.001	0.96	
versus Metformin				<0.001	0.99	
versus Syntra					<0.001	
MCSF (pg/mL)	5.9±1.8	6.7±0.8	8.9±2.5	5±3.1	9.3±2.4	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.99	0.79	0.44	0.73	
versus Byetta			0.96	0.26	0.94	
versus Metformin				0.05	1	
versus Syntra					0.04	
MIP-1 alpha (pg/mL)	22.9±20.7	28.4±7.4	33.3±25.5	27±16.8	16.4±5.9	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		1	0.44	1	0.23	
versus Byetta			0.54	1	0.12	
versus Metformin				0.57	0.004	
versus Syntra					0.11	

[1]F Test
[2]Based on a linear model of the logarithm of the values.
[3]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 3. Chemokine Levels by Treatment (Normal Diet Only)

	Treatment					
	Actos	Byetta	Metformin	Syntra-5	Untreated	P-Value
MIP-2 (pg/mL)	52.7±15.5	61.3±3.7	58.8±15.2	54.7±19.3	54.3±6.8	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.62	0.88	1	0.99	
versus Byetta			0.99	0.71	0.88	
versus Metformin				0.93	0.99	
versus Syntra					1	
RANTES (pg/mL)	25.9±13.3	42.1±18	41.2±18.4	29.9±6.7	28±21.4	0.27[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.37	0.35	0.89	1	
versus Byetta			1	0.87	0.26	
versus Metformin				0.87	0.24	
versus Syntra					0.79	
sRANKL (pg/mL)	31.6±27.4	23.3±4.7	33.5±12.3	23.2±7.9	26.5±6.3	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.98	0.82	0.93	1	
versus Byetta			0.52	1	0.98	
versus Metformin				0.35	0.84	
versus Syntra					0.91	

[1]F Test
[2]Based on a linear model of the logarithm of the values.
[3]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 4. Cytokine Levels by Treatment (Normal Diet Only)

	Treatment					
	Actos	Byetta	Metformin	Syntra-5	Untreated	P-Value
IL-17 (pg/mL)	66.2±41.5	133±53.5	144.5±127.7	104.6±54.8	118.9±69.8	<0.001[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.09	0.13	0.51	0.21	
versus Byetta			1	0.81	0.98	
versus Metformin				0.91	1	
versus Syntra					0.98	
IFN-Gamma (pg/mL)	56.2±95.7	30±71.1	134±349.9	116.4±202.4	188±493.8	0.8[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.99	0.96	0.99	1	
versus Byetta			1	1	0.97	
versus Metformin				1	0.89	
versus Syntra					0.92	
IL-1 beta (pg/mL)	95.7±31.6	106±12.8	129±47.9	100.7±15.9	119.1±32.3	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.93	0.67	0.98	0.29	
versus Byetta			0.99	0.67	0.79	
versus Metformin				0.33	0.97	
versus Syntra					0.1	

[1]F Test
[2]Based on a linear model of the logarithm of the values.
[3]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 4. Cytokine Levels by Treatment (Normal Diet Only)

	Treatment					
	Actos	Byetta	Metformin	Syntra-5	Untreated	P-Value
IL-1 alpha (pg/mL)	28.8±5.3	31±4.9	32.9±8	27.3±4.6	34.6±6.9	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.81	0.19	0.95	0.35	
versus Byetta			0.82	0.99	0.95	
versus Metformin				0.55	1	
versus Syntra					0.77	
IL-2 (pg/mL)	0±0	0±0	0±0	0±0	27.4±77.6	1[1]
P-Value[23] for pairwise comparison:						
versus Actos						
versus Byetta						
versus Metformin						
versus Syntra						
IL-3 (pg/mL)	3.8±8.5	8.1±16.1	12.9±31.5	8.9±11.2	13.2±33.6	0.02[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.8	0.94	0.33	1	
versus Byetta			1	0.93	0.86	
versus Metformin				0.76	0.97	
versus Syntra					0.36	

[1]F Test
[2]Based on a linear model of the logarithm of the values.
[3]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 4. Cytokine Levels by Treatment (Normal Diet Only)

	Treatment					
	Actos	Byetta	Metformin	Syntra-5	Untreated	P-Value
IL-4 (pg/mL)	1.7±2.2	2.4±0.8	1.1±1.3	2.6±2	0.8±0.5	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.71	1	0.58	0.66	
versus Byetta			0.92	1	0.07	
versus Metformin				0.85	0.46	
versus Syntra					0.04	
IL-5 (pg/mL)	8.5±5.1	9.4±3.7	10±5.8	8.3±4.9	5.9±2.2	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		1	0.83	1	0.95	
versus Byetta			0.96	0.97	0.84	
versus Metformin				0.69	0.42	
versus Syntra					0.99	
IL-6 (pg/mL)	9.8±8.1	11.4±2.5	30.8±59.4	13.7±9.9	17±12.9	0.16[1]
P-Value[23] for pairwise comparison:						
versus Actos		1	1	0.99	1	
versus Byetta			1	1	0.99	
versus Metformin				0.95	1	
versus Syntra					0.91	

[1]F Test
[2]Based on a linear model of the logarithm of the values.
[3]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 4. Cytokine Levels by Treatment (Normal Diet Only)

	Treatment					
	Actos	Byetta	Metformin	Syntra-5	Untreated	P-Value
IL-9 (pg/mL)	31.2±38.4	7.1±9.3	24±23.6	1.5±4.3	16.9±7.7	0.73[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.29	1	0.36	0.32	
versus Byetta			0.2	0.99	0.99	
versus Metformin				0.29	0.2	
versus Syntra					0.91	
IL-10 (pg/mL)	0±0	0±0	0±0	0±0	0±0	1[1]
P-Value[23] for pairwise comparison:						
versus Actos						
versus Byetta						
versus Metformin						
versus Syntra						
IL-12 p40 (pg/mL)	75.6±26.2	154.8±17.9	129.2±34.9	127.7±68.8	131±51.1	0.75[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.002	0.03	0.13	0.03	
versus Byetta			0.81	0.41	0.78	
versus Metformin				0.96	1	
versus Syntra					0.97	

[1]F Test
[2]Based on a linear model of the logarithm of the values.
[3]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 4. Cytokine Levels by Treatment (Normal Diet Only)

	Treatment					
	Actos	Byetta	Metformin	Syntra-5	Untreated	P-Value
IL-12 p70 (pg/mL)	44.4±33	59.9±9	51.7±23.3	52.4±27.9	40.9±25.4	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.8	1	1	0.98	
versus Byetta			0.91	0.83	0.47	
versus Metformin				1	0.92	
versus Syntra					0.97	
IL-13 (pg/mL)	3.9±4.7	7.3±6.2	7.5±9.8	8.7±5.5	9.3±18.1	0.37[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.5	0.56	0.66	0.88	
versus Byetta			1	1	0.95	
versus Metformin				1	0.97	
versus Syntra					0.99	
IL-23 (pg/mL)	7.8±11.1	13±10.2	26.6±30.6	27.5±25.3	18.8±12.1	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.66	0.34	0.39	0.58	
versus Byetta			0.99	1	1	
versus Metformin				1	0.99	
versus Syntra					1	

[1]F Test
[2]Based on a linear model of the logarithm of the values.
[3]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 4. Cytokine Levels by Treatment (Normal Diet Only)

			Treatment			
	Actos	Byetta	Metformin	Syntra-5	Untreated	P-Value
IP-10 (pg/mL)	616.3±486.2	572.1±445	1074.9±2178.9	1125.1±1506.2	1161.6±2405.2	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		1	1	0.99	0.99	
versus Byetta			1	0.99	1	
versus Metformin				0.96	1	
versus Syntra					0.91	

[1]F Test
[2]Based on a linear model of the logarithm of the values.
[3]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 5. Endocrine Levels by Treatment (Normal Diet Only)

			Treatment			
	Actos	Byetta	Metformin	Syntra-5	Untreated	P-Value
Adiponectin (ng/mL)	245.4±77.9	211.5±43.6	199.1±34.7	190.4±29	265±39.4	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.69	0.34	0.17	0.85	
versus Byetta			0.98	0.89	0.18	
versus Metformin				0.99	0.047	
versus Syntra					0.02	
Leptin (ng/mL)	32.1±15.4	28.1±4.4	36.5±30.4	30.3±14.8	43.7±10.8	0.001[1]
P-Value[23] for pairwise comparison:						
versus Actos		1	1	0.97	0.62	
versus Byetta			1	1	0.51	
versus Metformin				0.96	0.64	
versus Syntra					0.26	

[1]F Test
[2]Based on a linear model of the logarithm of the values.
[3]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 6. Growth Factor Levels by Treatment (Normal Diet Only)

			Treatment			
	Actos	Byetta	Metformin	Syntra-5	Untreated	P-Value
G-CSF (pg/mL)	29.5±25	20±7.8	225.2±530.8	73.2±87.2	44.3±40.1	<0.001[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.99	0.76	0.96	0.97	
versus Byetta			0.54	0.84	0.84	
versus Metformin				0.98	0.98	
versus Syntra					1	
GM-CSF (pg/mL)	63.1±80.3	94.8±144.4	160.6±350.5	182.4±256.6	226.6±535.2	0.01[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.99	0.99	0.81	0.98	
versus Byetta			1	0.97	1	
versus Metformin				0.96	1	
versus Syntra					0.98	
TGF beta (pg/mL)	29±21.4	34.4±10	41.8±40.7	54.8±25.6	83.6±74.9	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.87	0.85	0.23	0.04	
versus Byetta			1	0.8	0.35	
versus Metformin				0.82	0.37	
versus Syntra					0.93	

[1]F Test
[2]Based on a linear model of the logarithm of the values.
[3]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 6. Growth Factor Levels by Treatment (Normal Diet Only)

			Treatment			
	Actos	Byetta	Metformin	Syntra-5	Untreated	P-Value
TNF alpha (pg/mL)	7±6	8±3.8	11±13.5	13.3±11.7	12±2.4	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.99	0.8	0.35	0.4	
versus Byetta			0.97	0.63	0.7	
versus Metformin				0.95	0.98	
versus Syntra					1	
VEGF (pg/mL)	8.8±5.6	7.8±1.9	8.9±4.5	7±2.6	10.5±3	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.91	0.95	0.48	0.99	
versus Byetta			1	0.94	0.63	
versus Metformin				0.86	0.72	
versus Syntra					0.19	

[1]F Test
[2]Based on a linear model of the logarithm of the values.
[3]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 7. Metabolite Levels by Treatment (Normal Diet Only)

	Treatment					
	Actos	Byetta	Metformin	Syntra-5	Untreated	P-Value
Glucose (mg/dL)	153.7±80	126.1±15	131.7±20.2	128.6±19.8	111.7±12	<0.001[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.81	0.95	0.86	0.19	
versus Byetta			1	1	0.81	
versus Metformin				1	0.6	
versus Syntra					0.72	
Triglycerides (mM)	0.02±0.01	0.03±0.01	0.02±0.01	0.02±0.02	0.01±0.01	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.72	0.83	0.99	0.93	
versus Byetta			1	0.47	0.28	
versus Metformin				0.6	0.39	
versus Syntra					1	
HDL (µg/µL)	0.49±0.07	0.53±0.12	0.41±0.11	0.51±0.17	0.41±0.1	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.99	0.66	1	0.65	
versus Byetta			0.42	0.98	0.41	
versus Metformin				0.75	1	
versus Syntra					0.75	

[1]F Test
[2]Based on a linear model of the logarithm of the values.
[3]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 7. Metabolite Levels by Treatment (Normal Diet Only)

	Treatment					
	Actos	Byetta	Metformin	Syntra-5	Untreated	P-Value
LDL & VLDL (µg/µL)	0.13±0.03	0.14±0.04	0.15±0.02	0.11±0.05	0.12±0.03	0.07[1]
P-Value[23] for pairwise comparison:						
versus Actos		1	0.82	0.72	0.95	
versus Byetta			0.9	0.71	0.93	
versus Metformin				0.17	0.39	
versus Syntra					0.99	
ATP (nM)	527.6±448	704.5±487.2	165.1±81.4	533.7±495.8	3885.4±5860.4	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		1	0.78	1	1	
versus Byetta			0.58	0.99	0.97	
versus Metformin				0.82	0.9	
versus Syntra					1	
ADP (µM)	1.8±1.1	1.9±1	0.8±0.3	1.7±1.1	5.1±6.6	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		1	0.92	1	0.76	
versus Byetta			0.9	1	0.73	
versus Metformin				0.93	1	
versus Syntra					0.78	

[1]F Test
[2]Based on a linear model of the logarithm of the values.
[3]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 7. Metabolite Levels by Treatment (Normal Diet Only)

	Treatment					
	Actos	Byetta	Metformin	Syntra-5	Untreated	P-Value
Pyruvate Kinase (mU/mL extract)	1.8±1	1.2±0.7	1.7±0.8	2.1±0.9	0.7±0.9	0.01[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.99	1	1	0.03	
versus Byetta			0.98	0.96	0.1	
versus Metformin				1	0.03	
versus Syntra					0.02	
Citrate (mM)	0.47±0.31	0.56±0.17	0.79±0.21	0.4±0.24	0.21±0.19	0.002[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.86	0.33	1	0.07	
versus Byetta			0.91	0.79	0.008	
versus Metformin				0.26	<0.001	
versus Syntra					0.09	
Advanced Glycation End Product (mg/dL)	1.6±0.3	4.4±1.8	1.9±0.7	5.4±2.1	1.6±0.6	<0.001[1]
P-Value[23] for pairwise comparison:						
versus Actos		<0.001	0.9	<0.001	1	
versus Byetta			0.003	0.79	<0.001	
versus Metformin				<0.001	0.8	
versus Syntra					<0.001	

[1]F Test
[2]Based on a linear model of the logarithm of the values.
[3]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 7. Metabolite Levels by Treatment (Normal Diet Only)

			Treatment			
	Actos	Byetta	Metformin	Syntra-5	Untreated	P-Value
Insulin (ng/mL)	8.8±4.5	11.6±4.9	20.7±2.5	4.4±4.9	16.8±6	<0.001[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.91	0.1	0.04	0.26	
versus Byetta			0.36	0.004	0.73	
versus Metformin				<0.001	0.93	
versus Syntra					<0.001	

[1]F Test
[2]Based on a linear model of the logarithm of the values.
[3]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 8. Chemokine Levels by Treatment (High Fat Diet Only)

			Treatment			
	Actos	Byetta	Metformin	Syntra-5	Untreated	P-Value
BTC (pg/mL)	141.3±125.3	67.4±68.6	77.2±68.4	23.2±47.4	153.3±122.3	0.08[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.8	0.75	0.43	0.87	
versus Byetta			1	0.88	0.3	
versus Metformin				0.91	0.26	
versus Syntra					0.14	
Eotaxin (ng/mL)	3.8±1	2.9±0.5	3.5±1.6	2.3±0.6	3.8±2	0.27[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.6	0.97	0.11	1	
versus Byetta			0.86	0.64	0.77	
versus Metformin				0.2	1	
versus Syntra					0.17	
KC (pg/mL)	237.2±217.3	135.6±121.5	210±159.2	64.2±40.2	254.7±291.1	0.8[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.5	0.97	0.22	1	
versus Byetta			0.85	0.94	0.52	
versus Metformin				0.5	0.98	
versus Syntra					0.23	

[1]F Test
[2]Based on a linear model of the logarithm of the values.
[3]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 8. Chemokine Levels by Treatment (High Fat Diet Only)

			Treatment			
	Actos	Byetta	Metformin	Syntra-5	Untreated	P-Value
LIF (pg/mL)	30.8±15.2	30.2±10.1	52±40.4	24.9±11.4	21.2±13.3	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		1	0.76	1	0.68	
versus Byetta			0.91	0.97	0.48	
versus Metformin				0.62	0.11	
versus Syntra					0.89	
LIX (ng/mL)	8.2±3.3	6±2	4.4±1.9	6.6±2.8	8.4±6.9	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.95	0.46	0.99	0.76	
versus Byetta			0.87	1	0.99	
versus Metformin				0.82	0.99	
versus Syntra					0.97	
MCP-1 (pg/mL)	159±52	167.3±50.3	157±91.8	185.2±166.8	230.2±142.6	<0.001[1]
P-Value[23] for pairwise comparison:						
versus Actos		1	0.99	1	0.75	
versus Byetta			0.95	0.99	0.89	
versus Metformin				1	0.49	
versus Syntra					0.73	

[1]F Test
[2]Based on a linear model of the logarithm of the values.
[3]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 8. Chemokine Levels by Treatment (High Fat Diet Only)

			Treatment			
	Actos	Byetta	Metformin	Syntra-5	Untreated	P-Value
MCP-3 (pg/mL)	757.8±193.2	860.5±177.5	457±287.7	455.7±433.1	1216.2±1456.3	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.99	0.22	0.81	1	
versus Byetta			0.08	0.57	1	
versus Metformin				0.94	0.16	
versus Syntra					0.7	
MCSF (pg/mL)	7.4±3	10.6±16.5	5.5±2.5	6.4±1.5	9.8±4.1	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.93	0.88	1	0.93	
versus Byetta			1	0.98	0.5	
versus Metformin				0.97	0.42	
versus Syntra					0.9	
MIP-1 alpha (pg/mL)	18.5±11.4	19.6±12.7	34.4±31.6	196±433.2	7.3±6	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		1	0.88	0.89	0.78	
versus Byetta			0.96	0.96	0.63	
versus Metformin				1	0.27	
versus Syntra					0.32	

[1]F Test
[2]Based on a linear model of the logarithm of the values.
[3]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 8. Chemokine Levels by Treatment (High Fat Diet Only)

			Treatment			
	Actos	Byetta	Metformin	Syntra-5	Untreated	P-Value
MIP-2 (pg/mL)	65.1±17.3	66.9±26	62.1±11.4	49.7±9.8	58.5±15.2	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		1	1	0.36	0.91	
versus Byetta			1	0.34	0.9	
versus Metformin				0.5	0.98	
versus Syntra					0.82	
RANTES (pg/mL)	26.1±11.9	21.3±16.8	20.5±8.2	11.8±9.9	42.3±56.8	0.27[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.92	0.97	0.57	1	
versus Byetta			1	0.92	0.89	
versus Metformin				0.85	0.95	
versus Syntra					0.52	
sRANKL (pg/mL)	30.1±24.9	26.5±9	18±9	29.5±30.7	37.6±36.9	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		1	0.58	1	0.99	
versus Byetta			0.55	1	1	
versus Metformin				0.61	0.36	
versus Syntra					1	

[1]F Test
[2]Based on a linear model of the logarithm of the values.

Syntra-5 Mice Study (6 December 2010)

Table 9. Cytokine Levels by Treatment (High Fat Diet Only)

			Treatment			
	Actos	Byetta	Metformin	Syntra-5	Untreated	P-Value
IL-17 (pg/mL)	439±230	953.2±660.5	377.2±181.2	196.4±159.8	502±723.3	<0.001[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.25	1	0.09	0.98	
versus Byetta			0.13	<0.001	0.09	
versus Metformin				0.18	1	
versus Syntra					0.24	
IFN-Gamma (pg/mL)	577.2±593.8	201.4±259	325.8±378.7	82.1±183.5	426.3±511.5	0.8[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.13	0.99	1	0.83	
versus Byetta			0.38	0.85	0.58	
versus Metformin				1	0.99	
versus Syntra					1	
IL-1 beta (pg/mL)	122.6±20	124.1±55.6	130.2±41.2	96.9±56.7	150.9±154.7	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		1	0.63	0.62	0.81	
versus Byetta			0.6	0.59	0.78	
versus Metformin				1	1	
versus Syntra					0.99	

[1]F Test
[2]Based on a linear model of the logarithm of the values.
[3]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 9. Cytokine Levels by Treatment (High Fat Diet Only)

			Treatment			
	Actos	Byetta	Metformin	Syntra-5	Untreated	P-Value
IL-1 alpha (pg/mL)	43.2±20.3	53.1±48.4	29.6±8.3	28.6±8.4	32.6±12	1[1]
P-Value[2,3] for pairwise comparison:						
versus Actos		1	1	0.66	1	
versus Byetta			1	0.78	1	
versus Metformin				0.58	1	
versus Syntra					0.75	
IL-2 (pg/mL)	40.2±113.8	0±0	24.8±70.3	0±0	49.7±140.7	1[1]
P-Value[2,3] for pairwise comparison:						
versus Actos						
versus Byetta						
versus Metformin						
versus Syntra						
IL-3 (pg/mL)	52±46.5	22.8±19.6	22.7±19.4	12.6±25.8	43.2±51.1	0.02[1]
P-Value[2,3] for pairwise comparison:						
versus Actos		0.88	0.34	0.96	1	
versus Byetta			0.88	1	0.95	
versus Metformin				0.98	0.47	
versus Syntra					0.98	

[1]F Test
[2]Based on a linear model of the logarithm of the values.
[3]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 9. Cytokine Levels by Treatment (High Fat Diet Only)

			Treatment			
	Actos	Byetta	Metformin	Syntra-5	Untreated	P-Value
IL-4 (pg/mL)	1.6±1.6	1.7±1	3.2±3.9	2±2.2	0.7±0.6	1[1]
P-Value[2,3] for pairwise comparison:						
versus Actos		0.83	0.66	0.52	0.93	
versus Byetta			1	0.94	0.4	
versus Metformin				0.98	0.25	
versus Syntra					0.22	
IL-5 (pg/mL)	9.7±5.6	11.9±8.3	14.4±6.9	14±7.6	14.7±22.5	1[1]
P-Value[2,3] for pairwise comparison:						
versus Actos		0.99	0.82	0.98	1	
versus Byetta			0.97	1	0.95	
versus Metformin				0.99	0.64	
versus Syntra					0.91	
IL-6 (pg/mL)	40.5±17.1	82.6±69.4	45±22.4	27.4±28.8	117.8±124.5	0.16[1]
P-Value[2,3] for pairwise comparison:						
versus Actos		0.81	1	0.93	0.94	
versus Byetta			0.84	0.43	1	
versus Metformin				0.92	0.96	
versus Syntra					0.61	

[1]F Test
[2]Based on a linear model of the logarithm of the values.
[3]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 9. Cytokine Levels by Treatment (High Fat Diet Only)

			Treatment			
	Actos	Byetta	Metformin	Syntra-5	Untreated	P-Value
IL-9 (pg/mL)	23.1±25.3	8.8±11.4	2.4±5.9	14.6±26.9	18±22	0.73[1]
P-Value[2,3] for pairwise comparison:						
versus Actos		0.93	0.98	0.95	0.94	
versus Byetta			1	1	1	
versus Metformin				1	1	
versus Syntra					1	
IL-10 (pg/mL)	0±0	0±0	0±0	0±0	3.3±9.34	1[1]
P-Value[2,3] for pairwise comparison:						
versus Actos						
versus Byetta						
versus Metformin						
versus Syntra						
IL-12 p40 (pg/mL)	135.3±33.9	157±99.4	179.8±87.9	103.4±75.4	278±415.6	0.75[1]
P-Value[2,3] for pairwise comparison:						
versus Actos		1	0.95	0.61	0.95	
versus Byetta			0.97	0.57	0.97	
versus Metformin				0.24	1	
versus Syntra					0.25	

[1]F Test
[2]Based on a linear model of the logarithm of the values.
[3]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 9. Cytokine Levels by Treatment (High Fat Diet Only)

	Treatment					
	Actos	Byetta	Metformin	Syntra-5	Untreated	P-Value
IL-12 p70 (pg/mL)	61±24	95.9±97.6	86.8±64.1	47.5±31.7	138±255.1	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		1	0.96	0.9	1	
versus Byetta			0.99	0.78	1	
versus Metformin				0.56	0.97	
versus Syntra					0.88	
IL-13 (pg/mL)	22.4±13.7	16.6±13.1	20.3±12.4	8.9±13.2	26.5±44.1	0.37[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.94	1	0.05	0.87	
versus Byetta			0.98	0.21	1	
versus Metformin				0.08	0.93	
versus Syntra					0.29	
IL-23 (pg/mL)	5.2±6.6	22.1±36.1	15.7±15.6	15.9±15.6	8.9±16.6	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.89	0.85	0.78	1	
versus Byetta			1	1	0.99	
versus Metformin				1	0.98	
versus Syntra					0.95	

[1]F Test
[2]Based on a linear model of the logarithm of the values.
[3]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 9. Cytokine Levels by Treatment (High Fat Diet Only)

	Treatment					
	Actos	Byetta	Metformin	Syntra-5	Untreated	P-Value
IP-10 (pg/mL)	3212±3381.7	1313.5±1279.1	1862.4±1204.7	486.5±466.1	2362.3±1922.5	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.47	0.94	0.13	0.93	
versus Byetta			0.86	0.8	0.88	
versus Metformin				0.33	1	
versus Syntra					0.35	

[1]F Test
[2]Based on a linear model of the logarithm of the values.
[3]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 10. Endocrine Levels by Treatment (High Fat Diet Only)

	Treatment					
	Actos	Byetta	Metformin	Syntra-5	Untreated	P-Value
Adiponectin (ng/mL)	228.4±53.8	236.1±44.7	193±68.3	210.6±115.1	302±176.6	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		1	0.86	0.87	0.89	
versus Byetta			0.76	0.78	0.95	
versus Metformin				1	0.34	
versus Syntra					0.39	
Leptin (ng/mL)	20.8±10.1	9.7±2.9	29.8±31.7	18±9.9	15.9±12.3	0.001[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.15	0.98	0.95	0.57	
versus Byetta			0.051	0.66	0.91	
versus Metformin				0.76	0.28	
versus Syntra					0.98	

[1]F Test
[2]Based on a linear model of the logarithm of the values.
[3]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 11. Growth Factor Levels by Treatment (High Fat Diet Only)

	Treatment					
	Actos	Byetta	Metformin	Syntra-5	Untreated	P-Value
G-CSF (pg/mL)	252.9±189.7	1378.1±1507.8	135.2±91.9	56.2±74	735.8±796.6	<0.001[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.22	0.73	0.06	0.99	
versus Byetta			0.01	<0.001	0.48	
versus Metformin				0.46	0.42	
versus Syntra					0.02	
GM-CSF (pg/mL)	527.1±449.5	266.4±267.2	425.1±424.9	81.9±129.7	457.8±433.1	0.01[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.67	0.92	0.01	0.99	
versus Byetta			0.99	0.18	0.9	
versus Metformin				0.07	1	
versus Syntra					0.03	
TGF beta (pg/mL)	56.2±30.8	45.8±29.9	33±24	22.5±34.3	74.9±72.1	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.97	0.89	0.01	1	
versus Byetta			1	0.05	0.84	
versus Metformin				0.11	0.71	
versus Syntra					0.005	

[1]F Test
[2]Based on a linear model of the logarithm of the values.
[3]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 11. Growth Factor Levels by Treatment (High Fat Diet Only)

	Treatment					
	Actos	Byetta	Metformin	Syntra-5	Untreated	P-Value
TNF alpha (pg/mL)	10.4±5.6	15.3±8.8	28.5±31.6	4.2±6.4	15±7.9	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.99	0.85	0.42	0.99	
versus Byetta			0.98	0.24	1	
versus Metformin				0.11	0.98	
versus Syntra					0.24	
VEGF (pg/mL)	6.6±3.2	9.1±3	9.2±5.6	6.2±4.8	9.9±2.5	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.98	1	1	0.88	
versus Byetta			0.95	0.92	1	
versus Metformin				1	0.8	
versus Syntra					0.78	

[1]F Test
[2]Based on a linear model of the logarithm of the values.
[3]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 12. Metabolite Levels by Treatment (High Fat Diet Only)

	Treatment					
	Actos	Byetta	Metformin	Syntra-5	Untreated	P-Value
Glucose (mg/dL)	291.3±202.3	412.4±195.5	343.9±226.7	224±42.4	189.7±65.7	<0.001[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.51	0.97	1	0.87	
versus Byetta			0.86	0.44	0.1	
versus Metformin				0.93	0.52	
versus Syntra					0.96	
Triglycerides (mM)	0.02±0.01	0.02±0.01	0.02±0.01	0.01±0.01	0.02±0.01	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.99	0.41	0.82	0.92	
versus Byetta			0.19	0.97	0.68	
versus Metformin				0.07	0.89	
versus Syntra					0.36	
HDL (µg/µL)	0.51±0.18	0.43±0.18	0.46±0.09	0.57±0.17	0.43±0.08	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.7	1	0.98	0.93	
versus Byetta			0.89	0.4	0.99	
versus Metformin				0.88	0.99	
versus Syntra					0.69	

[1]F Test
[2]Based on a linear model of the logarithm of the values.
[3]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 12. Metabolite Levels by Treatment (High Fat Diet Only)

	Treatment					
	Actos	Byetta	Metformin	Syntra-5	Untreated	P-Value
LDL & VLDL (μg/μL)	0.15±0.04	0.14±0.05	0.24±0.1	0.19±0.08	0.22±0.12	0.07[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.99	0.21	0.84	0.59	
versus Byetta			0.08	0.57	0.3	
versus Metformin				0.85	0.95	
versus Syntra					1	
ATP (nM)	397.8±467.4	269.1±203.1	146.4±111.3	520.5±421.1	270.1±144.6	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		1	0.67	0.94	1	
versus Byetta			0.78	0.89	0.99	
versus Metformin				0.29	0.53	
versus Syntra					0.98	
ADP (μM)	1.5±1.1	1.4±0.7	0.9±0.4	2.2±1.3	1.6±0.7	1[1]
P-Value[23] for pairwise comparison:						
versus Actos		1	0.75	0.64	0.89	
versus Byetta			0.59	0.78	0.96	
versus Metformin				0.11	0.23	
versus Syntra					0.98	

[1]F Test
[2]Based on a linear model of the logarithm of the values.
[3]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 12. Metabolite Levels by Treatment (High Fat Diet Only)

	Treatment					
	Actos	Byetta	Metformin	Syntra-5	Untreated	P-Value
Pyruvate Kinase (mU/mL extract)	2.1±1.3	2.1±1.7	3.2±3.1	2±0.8	1.6±0.8	0.01[1]
P-Value[23] for pairwise comparison:						
versus Actos		1	0.86	1	0.98	
versus Byetta			0.83	1	0.99	
versus Metformin				0.97	0.53	
versus Syntra					0.94	
Citrate (mM)	0.48±0.31	0.63±0.32	0.62±0.34	0.63±0.16	0.22±0.27	0.002[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.94	0.97	0.84	0.05	
versus Byetta			1	1	0.008	
versus Metformin				0.99	0.01	
versus Syntra					0.007	
Advanced Glycation End Product (mg/dL)	2.5±1.9	2.8±2.5	1.3±0.2	2.4±1.8	1.7±0.8	<0.001[1]
P-Value[23] for pairwise comparison:						
versus Actos		1	0.54	1	0.84	
versus Byetta			0.36	1	0.67	
versus Metformin				0.64	0.98	
versus Syntra					0.9	

[1]F Test
[2]Based on a linear model of the logarithm of the values.
[3]Tukey's adjustment for multiple testing used.

Syntra-5 Mice Study (6 December 2010)

Table 12. Metabolite Levels by Treatment (High Fat Diet Only)

	Treatment					
	Actos	Byetta	Metformin	Syntra-5	Untreated	P-Value
Insulin (ng/mL)	4.4±3.6	1.4±0.4	4.6±4.1	3.5±1.3	3±4.6	<0.001[1]
P-Value[23] for pairwise comparison:						
versus Actos		0.76	1	1	0.6	
versus Byetta			0.77	0.73	1	
versus Metformin				1	0.57	
versus Syntra					0.55	

[1]F Test
[2]Based on a linear model of the logarithm of the values.
[3]Tukey's adjustment for multiple testing used.

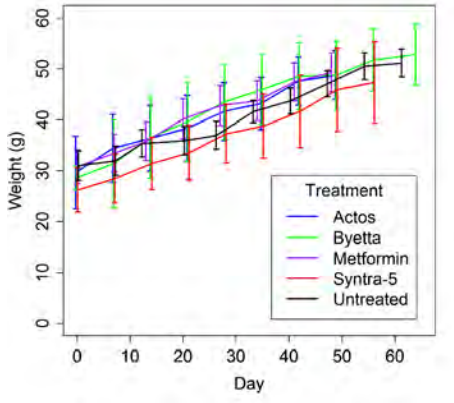


Figure 1.

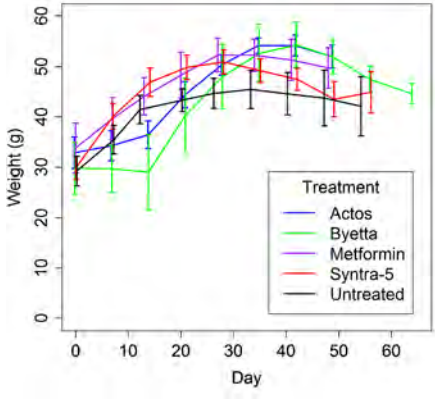


Figure 2.

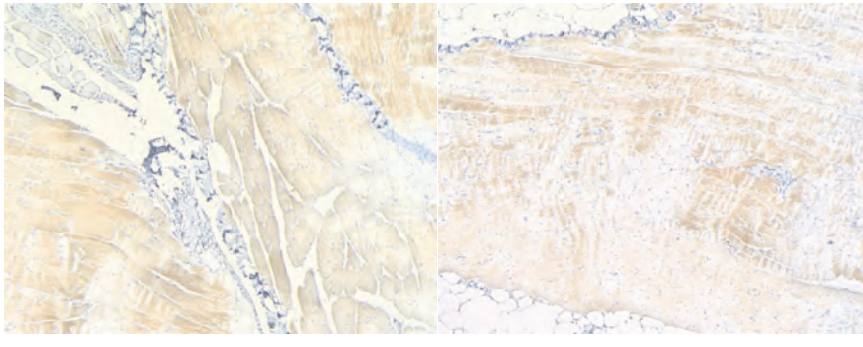


Figure 3A.

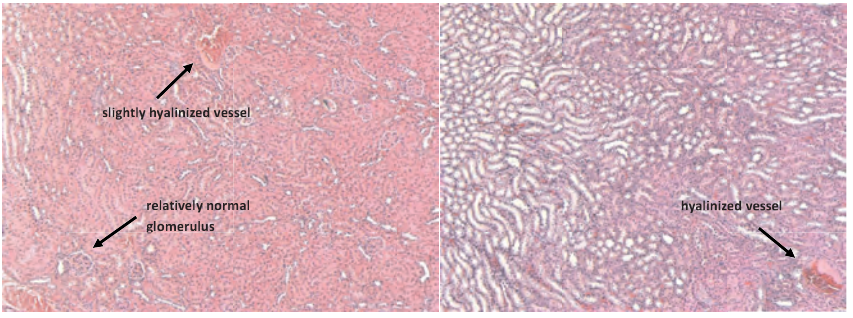


Figure 3B.

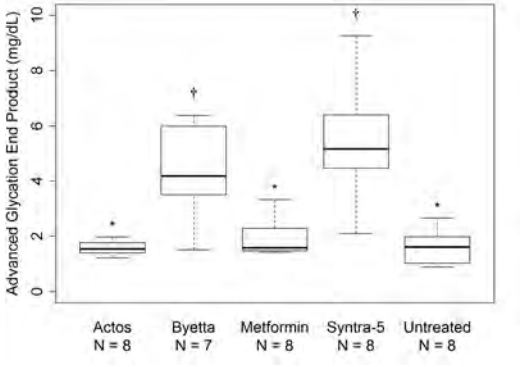


Figure 4.

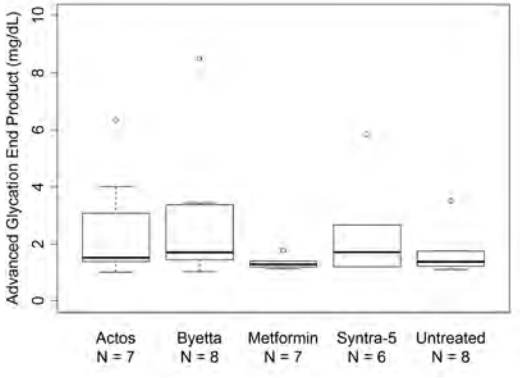
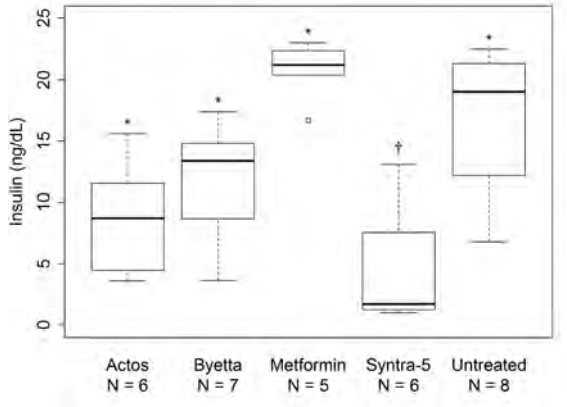


Figure 5.



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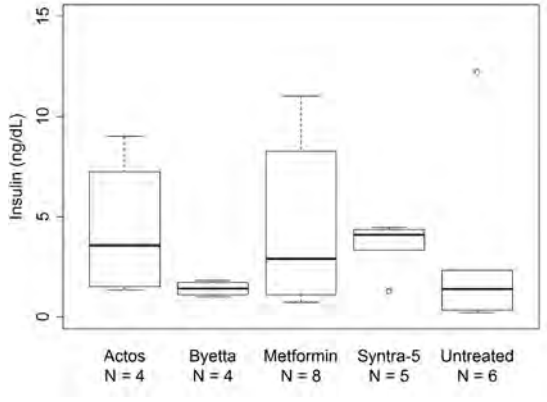

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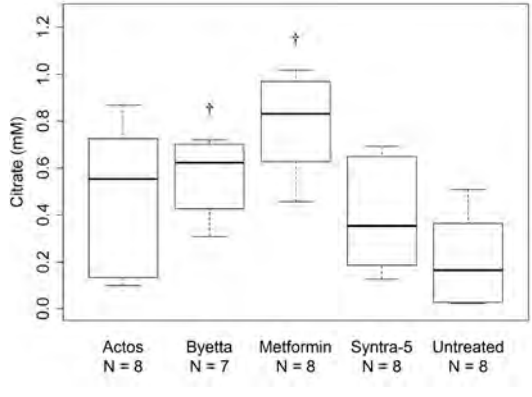

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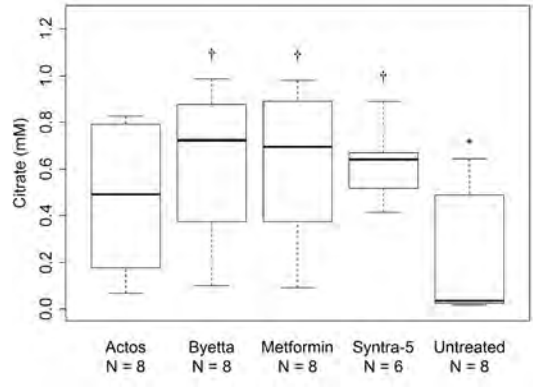

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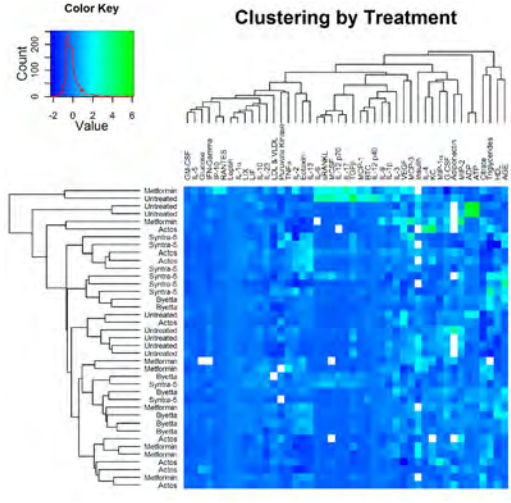

Figure 10.

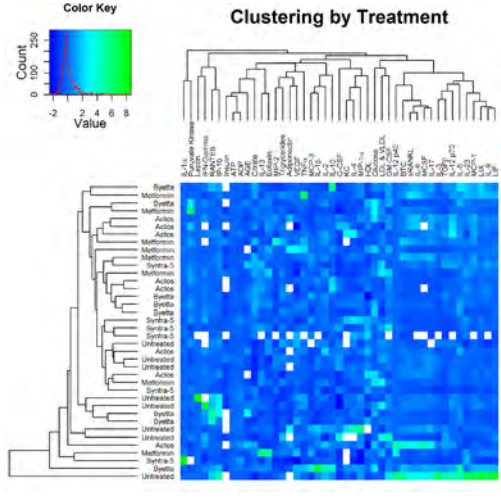

Figure 11.