WEIGHT LOSS IN OBESE SUBJECTS WITH AND WITHOUT TYPE 2 DIABETES TREATED WITH THE LONG TERM APPETITE SUPPRESSANT AXOKINE®

By

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Abstract of the Thesis

WEIGHT LOSS IN OBESE SUBJECTS WITH AND WITHOUT TYPE 2 DIABETES TREATED WITH THE LONG TERM APPETITE SUPPRESSANT AXOKINE ®

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Studies comparing weight loss between obese or overweight individuals with and without type 2 diabetes show a decreased weight loss in those with type 2 diabetes. The exact mechanisms promoting this discrepancy are unclear but may be related to the type and amount of antidiabetes medication, an altered metabolism, stage of disease, and/or presence of dysphoria. In this study, retrospective weight loss data from two double-blind, placebo controlled, randomized studies using the long-term appetite suppressant AXOKINE (a modified ciliary neurotrophic factor) were compared. In the first study, a total of 179 obese, nondiabetic subjects received placebo or AXOKINE at a 0.3µ/kg, 1.0 µg/kg, or 2.0 µ/kg dose for 12 weeks. In the second study, 142 obese subjects with type 2 diabetes received placebo, 0.5µg/kg or 1.0 µg/kg dose of AXOKINE for 12 weeks. Nutritional counseling was similar for both groups however the diabetic group received more frequent sessions. Mean weight loss between the two groups was similar with diabetic subjects losing 3.0 $\pm$ 2.6 kg vs. 3.4 $\pm$ 2.5 kg in the nondiabetic group (1.0 µg/kg

dose of AXOKINE). The subjects with diabetes did not have any significant improvements in HbA_{1c} (-0.34 $\pm$ 0.89%) or blood sugar levels (-15.5 $\pm$ 37.3 mg/dL) from baseline. Diabetic subjects also suffered more from diagnosed co-morbidities (55% of subjects had hypertension and 55% were dyslipidemic) and were likely to be on multiple oral hypoglycemic agents (66% of subjects). The amount of oral hypoglycemic agent did not appear to affect weight loss as those on multiple medications lost a similar amount of weight than the mean weight loss achieved by the nondiabetic subjects (3.3 $\pm$ 3.1 kg in the diabetic, multiple medication group vs. 3.4 $\pm$ 2.5 kg in the nondiabetic group). In contrast to the hypothesis that diabetic patients would lose less weight with and without AXOKINE compared to nondiabetic counterparts, these results suggest that weight loss is similar over a 12-week time period. A difference in weight loss may have been observed however with a longer study period.

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List of Abbreviations

ADA	**American Diabetes Association**
ALS	**Amyotrophic Lateral Sclerosis**
BMI	**body mass index**
CNTF	**ciliary neurotrophic factor**
DCCT	**Diabetes Control and Complications Trial**
FDA	**Food and Drug Administration**
GLP-1	**glucagon-like peptide-1**
HbA_1c	**glycated hemoglobin**
HMO	**health maintenance organization**
IRB	**Institutional Review Board**
ITT	**intend to treat**
Kg	**kilograms**
NHLBI	**national heart, lung and blood institute**
NIH	**national institute of health**
TZD's	**thiazolidinediones**
µg/kg	**microgram per kilogram**
UKPDS	**U.K. prospective diabetes study**
WHO	**World Health Organization**

1 Introduction

Type 2 diabetes mellitus currently affects approximately sixteen million people in the U.S. (Mahler 1999). An additional 30 to 40 million people have impaired blood glucose tolerance and will likely become diabetic. The estimated total annual health care cost for type 2 diabetes is staggering at 100 billion dollars (Lewin 1999). Sixty-one percent of those diagnosed with diabetes are obese (Bloomgarden 2003). In the U.S., half of the adults are now considered overweight or obese and obesity has quadrupled in teenagers (Bloomgarden 2003). Adults with a BMI of $\geq$ 35 have an increased risk of approximately 20 times of developing diabetes over a 10 year period than their peers who have a BMI between 18.5 and 24.9 (Field 2001). In addition to diabetes, obesity has been identified as a major risk factor for cardiovascular disease, hypertension, dyslipidemia, sleep apnea, musculoskeletal disorders and some cancers (NHLBI 1998).

Lifestyle changes such as a healthy diet for weight loss and increased physical activity are first line recommendations for patients newly diagnosed with type 2 diabetes. In obese patients with impaired glucose tolerance, weight loss as little as 5% has been shown to be sufficient to reduce the risk of type 2 diabetes (Astrup 2000). Exercise in addition to weight loss can help treat and prevent type 2 diabetes as research indicates that physical activity or energy expended from walking and vigorous activity can reduce risk of diabetes in a dose-dependent fashion (Wing 2001).

Previous studies indicate that the presence of type 2 diabetes in an obese individual may obstruct weight loss efforts as these patients have been shown to either lose weight more slowly and/or regain weight more quickly than non diabetic overweight or obese individuals (Guare 1995, Wing 1987)

1.1 Obesity and Weight Loss

On average, an overweight or obese person will lose about 10 percent (20 lb) of their weight by participating in a structured program offering behavioral modification, exercise, and a diet plan for 20 -26 weeks. After one year, patients have typically regained ~30 percent of their initial weight loss and most return to their baseline weight within three to five years. The most reliable predictors of long-term maintenance of weight loss are increased physical activity and devotion to self-monitoring (Wing 2001). The NWCR (National Weight Control Registry) has registered more than 6,000 people who have lost 30 pounds or more and have kept if off for at least a year. People who have successfully maintained their weight loss indicate that they eat a low-fat diet, watch their total calories and do a lot of physical activity (NIH 2006). Overweight or obese people face many hurdles to weight loss. A sedentary lifestyle has become widely acceptable, there has been a decline in traditional lifestyles, the medical profession not only lacks the incentives (e.g. financial) to help a patient lose weight, but also the knowledge, plus foods that are overly refined and high in fat are cheap and readily available. Even if an obese patient seeks pharmaceutical aid, the approved medications are not that effective and have many side effects (Kelner 2003). Data made available from the NHANES and the Coronary Artery Risk Development in Young Adults (CARDIA) study indicates that in the rate of weight gain over eight years among subjects 20 to 40 years old is 1.8 to 2.0 pounds/year. On the basis of this estimate, Hill et al. calculated an energy gap (required change in energy expenditure relative to energy intake necessary to restore energy balance) in the population to be 50 kilocalories/day. Since energy is stored with an efficiency of at least 50% for almost everyone, an excess of 100

kilocalories/day would theoretically provide 50 kilocalories to be deposited in energy stores. Thus, if individuals were to expend an extra 100 kcal/day (walking an extra mile) or by eating less, weight gain could be prevented (Hill 2003).

1.2 Currently Approved Medications for Weight Loss

Medications currently approved for weight loss in the U.S. can be placed in two broad categories: those that reduce food intake by decreasing appetite or increasing satiety and those that reduce nutrient absorption. A third category could be medications that increase energy expenditure (e.g.,ephedrine) however, none has been approved for treatment of obesity in the U.S. (Yanovski 2002).

Appetite suppressants mainly function by increasing norepinephrine, serotonin, dopamine, or some combination of these neurotransmitters in the central nervous system. Noradrenergic (stimulated by or releasing norepinephrine) drugs that fall into this category are phentermine, diethylpropion, phendimetrazine, and benzphetamine. All of these medications are approved by the FDA for only a "few week" use (usually presumed to be 12 weeks or less) for the treatment of obesity. A few anti-obesity drugs, including the fenfluramines and orlistat have produced weight loss in excess of 10 percent in 20 – 30 percent of obese type 2 diabetic patients with concomitant improvements in glycemic control (McNulty, 2003). Other medications such as serotonogeric compounds act by inhibiting the reuptake of serotonin, increasing its release, or both. Fenfluramine (Pondimin) and dexfenfluramine (Redux) are medications that induced both actions however these drugs were withdrawn for the U.S. market in 1997 after they were associated with valvular heart disease and pulmonary hypertension (Yanovski 2002).

1.2.1 Sibutramine

A mixed noradrenergic-serotonergic agent such as Meridia (sibutramine) is an inhibitor of both serotonin reuptake and norepinephrine reuptake. Meridia also inhibits dopamine reuptake at a low level and is approved by the FDA for weight loss and weight maintenance concomitant with a reduced calorie diet. It is estimated that over a six month period, those who follow a reduced-calorie diet and take sibutramine will lose up to 5 to 8 percent of their preintervention body weight. Weight loss induced by sibutramine has been observed to be maintained for periods of up to one year. Side effects observed with sibutramine administration include increased blood pressure and pulse rate, dry mouth, headache, insomnia and constipation (Yanovski 2002).

1.2.2 Orlistat

Xenical (orlistat), the only FDA-approved medication for obesity, reduces nutrient absorption, and prevents hydrolysis of dietary fat into absorbable free fatty acids and monoacylglycerols. In double-blind, placebo-controlled trials, orlistat-treated subjects lost approximately 9 percent of their preintervention body weight after one year of treatment. Unfortunately, orlistat treated subjects also had a slow regain of weight during the second year of use (regain of 35.2% orlistat vs. 62.4% placebo). Adverse effects of orlistat administration include fecal urgency, fecal incontinence, flatulence with discharge, steatorrhea, oily spotting and increased frequency of defecation (Yanovski 2002).

1.3 Epidemiology of Type 2 Diabetes Mellitus

Type 2 diabetes is a multisystem disease which is associated with insulin resistance, elevated blood glucose levels and other metabolic abnormalities such as dyslipidemia. Common long-term complications in these subjects are caused by pathological changes of the macro- and microvasculature. Stroke prevalence is two to six times higher in subjects with type 2 diabetes while cardiovascular disease is increased by two to four times and causes 75% of all diabetes related deaths (ADA 2002). Additionally, there are diabetes-specific complications which include diabetic retinopathy, kidney failure, and neuropathy. Diabetic foot ulcers are another disabling complication which may require amputation (Mahler 1999).

The American Diabetes Association has specified a set of main criteria for diagnosing type 2 diabetes mellitus: symptoms of diabetes and a casual plasma glucose of 200 mg/dl (11.1 mmol/l) - the classic symptoms of diabetes include polyuria, polydipsia, and unexplained weight loss; fasting plasma glucose $\geq$ 126 mg/dl (7.0 mmol/l) - fasting is defined as no caloric intake for at least 8 hours; a 2-hour plasma glucose of 200 mg/dl (11.1 mmol/l) during an oral glucose tolerance test - the test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of 75 grams anhydrous glucose dissolved in water (ADA 2006).

The development of diabetes can have several pathogenic origins ranging from autoimmune destruction of the B-cells of the pancreas, resulting in insulin deficiency, to abnormalities leading to resistance to insulin action. Overall insulin activity is decreased from a reduction in insulin secretion and/or a reduced tissue response to insulin. Insufficient insulin action on target tissues causes abnormalities in carbohydrate, protein

and fat metabolism. One patient can suffer from an impairment in insulin secretion as well as defective insulin activity, thus obscuring which abnormality, if either one is to be acting alone, is responsible for the hyperglycemia (Expert Committee 2003).

Modest weight loss in obese patients with type 2 diabetes is associated with improvements in glycemic control, insulin levels and lipid profile (Fujioka 2000). Glycemic control is quintessential for the management of diabetes. Improved glycemic control has been shown in the Diabetes Control and Complications Trial (DCCT) and the U.K. Prospective Diabetes Study (UKPDS) to be associated with lower sustained rates of nephropathy, retinopathy, and neuropathy. Subjects experiencing a drop in their HbA_1c to 7% (1% above the upper limits of normal) had a reduction in long term microvascular complications (Astrup 2000).

1.4 Currently Approved Medications for Type 2 Diabetes

Currently approved treatments for the control of the metabolic abnormalities observed in type 2 diabetes include anti-diabetic and lipid lowering drugs. Patients may be initially treated with diet and oral drugs such as sulfonylureas, metformin, and thiazolidinediones.

1.4.1 Thiazolidinediones

Thiazolidinediones (TZD's) are known to improve insulin sensitivity and glycemic control in patients with type 2 diabetes and also for those who are obese (Hallakou 1997). Thiazolidinediones have been associated with a small weight gain when used as monotherapy for 12 weeks or longer and larger gains have been observed when TZD's are combined with insulin or sulfonylurea (DeFronzo 1999). The increase

in weight is thought to be from increased fluid retention and an increase in fat mass. One of the factors behind an increase in fat mass is an increase in the number of small fat cells. These new, smaller fat adipocytes have a higher lipid storage capacity and are more insulin sensitive. The characteristics of the remodeled adipose tissue likely serves to lower free fatty acid levels and levels of skeletal muscle triglyceride - which may account for the reduction in skeletal muscle insulin resistance (De Souza 2001, Sugiyama 1990).

1.4.2 Sulfonylureas

Sulfonylureas stimulate insulin release from pancreatic beta-cells. All sulfonylureas have been associated with weight gain and many types of sulfonylureas are frequently associated with hypoglycemia (Feingloss 1999). A third generation sulfonylurea called "glimepride" has been observed to cause less hypoglycemia and less weight gain than the first generation medication (Rosak 2002). After an average of 10 years of use, most patients treated with sulfonylureas will require a second oral agent (DeFronzo 1999).

1.4.3 Biguanides

Biguanides (e.g., metformin) lower fasting blood glucose concentrations and increase peripheral and hepatic insulin sensitivity by inhibiting hepatic gluconeogenesis. Metformin is also not associated with hypoglycemia or weight gain or and in some studies, has been linked to weight loss (Feinglos 1999). Along with TZD's, biguanides do not directly stimulate insulin release.

1.4.4 Alpha-glucosidase Inhibitors

Alpha-glucosidase inhibitors (e.g., acarbose) also do not stimulate insulin release. Acarbose interferes with the digestion of dietary glucose precursors and the absorption of glucose. Alpha-glucosidase inhibitors can provide short term glycemic control and have been shown to reduce HbA_{1c} levels (Hanefeld 1998). Alpha-glucosidase inhibitors such as Acarbose are not associated with hypoglycemia or weight gain (Feinglos 1999).

1.4.5 Meglitinides

Meglitinides (e.g., repaglinide) and D-phenylalanine derivatives (e.g., nateglinide) are powerful insulin secretagogues (Rosack 2002). Repaglinide is not known to have any significant effect on plasma lipid levels and it has been observed that in subjects changing from sulfonylurea therapy to repaglinide treatment, no weight was gained (DeFronzo 1999). In patients new to drug therapy, however, repaglinide resulted in weight gain of approximately 3% (5 to 6 pounds) (Marbury 1999).

1.4.6 Insulin

Patients are often prescribed insulin when their diabetes is difficult to control with diet, exercise, and oral hypoglycemic agents. It has been observed repeatedly that insulin administration causes weight gain. This has been apparent when insulin is introduced after the failure of oral agents and also when insulin is given shortly following the diagnosis of diabetes. After 10 years of study in the UKPDS trial, patients treated with insulin gained 2 kg more than patients treated with sulfonylureas (Larger 2001).

1.5 Type 2 Diabetes and Weight Loss

It has been observed that people with type 2 diabetes have more difficulty losing weight and/or maintaining weight loss than nondiabetic individuals (Miles 2002, Wing 1987, Guare 1995). The mechanisms postulated for this discrepancy are behavioral, biochemical and progression of disease. Few studies have compared weight loss between age-, weight- and sex matched controls in obese diabetic and obese nondiabetic groups.

1.5.1 Previous Research Studies on Weight Loss and Type 2 Diabetes

A study performed by Wing et al. compared weight loss between obese diabetic subjects and their nondiabetic spouses. Twenty-four subjects (12 men, 12 women) participated in a 20-week behavioral weight control program consisting of self monitoring, stimulus control, cognitive restructuring, and contingency contracting. The diabetic subject group and the nondiabetic subject group each consisted of 6 men and 6 women. At the end of study period, the nondiabetic group lost significantly more weight (13.4 $\pm$ 1.7 kg vs. 7.5 $\pm$ 1.4 kg.) than their diabetic spouses. Although there was not a significant difference in exercise, the nondiabetic subjects were able to reduce their daily calorie intake by 1172 calories, while diabetic spouses only reduced their intake by 709 calories/day. The discrepancy in weight loss was associated with a higher incidence of depression in the diabetic group, as it may have led to a higher calorie consumption than the nondiabetic spouses (Wing 1987).

A study done by Guare JC, et al. comparing weight loss in 20 diabetic and 23 nondiabetic women treated with the same 16-week behavioral weight loss program revealed that subjects with diabetes regained more weight (5.4 $\pm$ 6.1 kg compared to 1.0

± 6.7 kg for nondiabetics) during a one year follow-up period. It was concluded that the subjects with type 2 diabetes required more follow up care to protect their weight loss and/or the weight re-gain may have been related to several other factors (e.g. changes in diabetes medications, self-reporting of post-treatment weights) (Guare 1995).

A similar tendency but a different explanation is offered in a retrospective study conducted by Khan et al, whereby 19 obese subjects (at least 30% over estimated ideal body weight) with type 2 diabetes and 19 obese subjects without diabetes were put on reduced calorie diets and provided with fen/phen (d.l-fenfluramine 20 mg by mouth TID and phentermine 30 mg by mouth daily). Each study subject was retrospectively matched to the first identified, nondiabetic subject, which was of the same gender, age (± 10%), and BMI (± 10%) before weight loss treatment. Subjects were also grouped based on therapy required for control of diabetes at the beginning of the weight loss treatment. The mean weight loss was 19.3% in nondiabetics vs. 11.2% in subjects with type 2 diabetes (Khan 2000). This difference in weight loss was associated with the potential presence of glycosuria and/or dissimilar appetite regulation in the patients with diabetes (Khan 2000).

Three well controlled studies using orlistat (a gastrointestinal lipase inhibitor) show a clear discrepancy in weight loss between obese patients with and without diabetes. Orlistat typically produces a weight loss of nine percent in obese nondiabetic individuals however, data from their 1- year, randomized, controlled trials in diabetic patients treated with either sulfonylurea or metformin therapy reveals that orlistat produced a smaller weight loss in these individuals (Kelley 2002). In three multicenter trials of orlistat therapy in type 2 diabetes, weight loss at 6 months was ~6% among sulfonylurea treated volunteers, ~4% among insulin treated volunteers and ~5% among those receiving

metformin therapy, and in each of the above trials, weight loss was generally 2-4% greater with orlistat than placebo (Kelley 2004). The relative difference in weight loss between the orlistat and placebo treatment groups seems to be consistent in diabetic and nondiabetic patients (Kelley 2002).

1.5.2 Alterations in Energy Expenditure

A reduction in energy expenditure preceded by enhanced treatment in diabetic patients seems counterintuitive, however poorly controlled patients with type 2 diabetes have been found to suffer from an increase in energy expenditure, increased protein turnover and glycosuria. Previously analyzed retrospective data for 24-hour whole body calorimetry measurements on subjects with type 2 diabetes and without showed that the 24-hour energy expenditure was 6.5% higher in the type 2 diabetic group (Bitz 2004). Other studies have shown a ~5% higher resting energy expenditure in Pima Indians with type 2 diabetes (Bogardus 1986, Fontvieille 1992, Weyer 1999) and in a single study in Caucasians (Franssila-Kallunki 1992). Additionally, urinary glucose excretion has been reported to be 3.1% of 24-h energy expenditure (103 $\pm$ 112 kcal/day) for obese individuals with type 2 diabetes receiving treatment (Weyer 2001). This estimation would be expected to increase if these patients discontinued their treatment (Bitz 2004).

1.5.3 Increased Free Fatty Acids

Free fatty acid levels are known to have a critical role in the pathogenesis of type 2 diabetes. Both glucose metabolism and insulin may be adversely affected by free fatty acids. Obese people have a higher free fatty acid flux. This can lead to peripheral insulin resistance and reduced insulin secretion as high levels of free fatty acids can interfere

with insulin signaling in muscle and have toxic effects on pancreatic-B cells. Increased flux of free fatty acids into the liver can increase hepatic glucose production (Pankow 2004). As described by David Kelley (Pittsburgh, PA), when persons with obesity combine weight loss with a fitness program, oxidative capacity increases, circulating free fatty acids are reduced, mitochondrial size increases, and the cytokine tumor necrosis factor (TNF-a) decreases. Additionally, skeletal muscle fat utilization capacity increases (Bloomgarden 2003).

1.5.4 Glucagon-like Peptide-1

Glucagon–like peptide-1 (GLP-1) is a small peptide secreted from the duodenum and distal bowel, respectively. GLP-1 is known to decrease glucagon secretion, gastric emptying, and food intake and promote B-cell growth. It has also been shown to increase satiety and reduce food intake in experimental animals and humans. Individuals with impaired glucose tolerance (IGT) have low levels of GLP-1 and those with type 2 diabetes have even less (Bloomgarden 2003). Studies looking at the effect of GLP-1 analogs (eg, pramlintide, liraglutide) in diabetes, have demonstrated that when given as adjunct therapy to insulin and/or oral hypoglycemic agents, they can improve glycemic and weight control in patients with type 2 diabetes (Hollander 2003, Degn 2004).

1.6 AXOKINE as a Long Term Appetite Suppressant

AXOKINE evolved from recombinant CNTF (ciliary neurotrophic factor). The primary role of CNTF is as a lesion factor released by the ruptured glial cells that prevent neuronal degradation. CNTF was initially tested to assess the effect on the symptoms in patients with amyotrophic lateral sclerosis (ALS) (ALS-CNTF Treatment Study, Phase I-

II, 1995). In a study conducted in over 500 subjects exposed to CNTF for up to nine months, the progression of ALS was not slowed however weight loss was reported in approximately 7% in patients receiving CNTF compared with placebo.

AXOKINE was engineered by introducing three specific modifications into CNTF: two modifications involved amino acid substitutions, and the third modification was the deletion of 15 non-essential amino acids from the C-terminus. AXOKINE was tested in a Phase I randomized, double blind, placebo controlled, parallel design study with two dose regimen cohorts. The first group received single, rising subcutaneous (injected beneath the skin) doses of AXOKINE or placebo. The second group received multiple, rising, daily subcutaneous doses of AXOKINE or placebo. A total of 52 normal volunteers with a BMI of 28 – 40 kg/m^2 received AXOKINE for 14 days. Participants were provided with a liberal diet without caloric restrictions and food intake was closely monitored. Over the two week treatment period, there was a gradual and dose dependent reduction in 24-hour food consumption ranging from -4% for placebo subjects to -47% in the subjects treated with AXOKINE at 4µg/kg/day. There was also a reduction in mean body weights in all of the active drug treatment arms (PL, 0.5 µg/kg, 1.0 µg/kg, 2.0 µg/kg, and 4.0 µg/kg), with a 1.8% decrease in weight for the 2.0 µg/kg/day dose group compared with baseline. AXOKINE was well tolerated at lower doses however dose limiting toxicities were persistent cough and asymptomatic increases in heart rate. Injection site reactions noted to be reddish spots up to 4 x 6 cm in size, were reported in all subjects although the incidence in drug treated subjects was significantly higher.

Similar to leptin, administration of CNTF into the venous circulation, caused a dose-dependent reduction in food intake in obese, *ob/ob* (lacking the gene to make leptin)

mice resulting in decreased body weight and adiposity (Sleeman 2003). CNTF has also been shown to reduce food intake and body weight in "leptin-resistant" forms of obesity, like diet-induced obesity (Gloaguen 1997, Lambert 2001). Contrary to these results, other studies show that AXOKINE treatment of *db*/*db* (loss of functional leptin receptor) mice caused significantly more weight loss than could be accounted for by reduced food intake alone and, instead, correlated with marked increases in metabolic rate and energy expenditure. Administration of AXOKINE also produced marked improvements in diabetic parameters [e.g., levels of glucose, insulin, triglyceride, cholesterol, and nonesterified free fatty acids (NEFA)], beyond that achieved by either equivalent weight loss or equivalent food restriction (Sleeman 2003).

Leptin, an adipocyte hormone, is primarily secreted by white adipose tissue. Since its discovery in 1994, leptin has been shown to have a wide range of effects on many different types of cells and its role in body weight maintenance is critical. Leptin interacts with hormonal mediators and regulators of energy status and metabolism such as growth hormone, insulin, insulin-like growth factors, glucagon and glucocorticoids (Margetic 2002). Concentrations of leptin in the blood are directly proportional to adipose tissue mass and are modulated by food intake (Frederich 1995, Nagy 1997, Rosenbaum 1996). Preclinical studies show that mice treated with leptin lost more weight than pair fed animals, suggesting that leptin may increase energy expenditure (Margetic 2002).

The CNTF receptor complex is closely related in functionality to the leptin receptor. The two receptors have overlapping distribution in the arcuate nucleus of the hypothalamus which is the key brain area for regulation of food consumption. The

glycoprotein gp130B, a component of the tripartite CNTF receptor complex, demonstrates structural homologies to ObR, exhibiting that binding of leptin or CNTF to their respective receptors activates similar signal transduction pathways. Thus, the administration of CNTF or leptin activates receptors in the arcuate nucleus of the hypothalamus and suppresses the expression of orexigenic peptides (peptides that promote weight gain), like neuropeptide Y and agouti-related peptide (Sleeman 2003).

2 Rationale and Hypothesis

Do overweight or obese people with type 2 diabetes have more difficulty losing weight than nondiabetic people? If yes, what are the reasons? It has been postulated that individuals with type 2 diabetes mellitus have altered biochemical processes and subsequent affected metabolism that hinders weight loss. Other effects could be physiological, treatment based (e.g. type of counseling/education received), or related to a rapid course of diabetes (Khan 2000). Deciphering how individuals with type 2 diabetes mellitus respond to weight loss methods can promote interventions leading to long term success. Studies conducted in subjects with type 2 diabetes have focused on the effect of anti-diabetic medication or appetite suppressant therapy on weight loss. Few studies, however, have compared weight loss or maintenance of weight lost between groups with type 2 diabetes and similar matched controls.

The type and amount of antidiabetic medication has been thought to hinder weight loss efforts. Insulin administration has been observed repeatedly to cause weight gain and it is common for newly diagnosed individuals with type 2 diabetes to gain approximately five pounds during initial oral hypoglycemic treatment with agents such as thiazolidinediones and sulfonylureas. Additionally, as the course of treatment ensues, energy expenditure may decrease through the reduction in glycosuria as the patient no longer loses large amounts of glucose through via urination (Hallakou 1997).

Increased energy expenditure, increased free fatty acid flux and lower levels of glucagon-like peptide-1 have been identified in obese people with type 2 diabetes (Bitz 2004, Bloomgarden 2003). There is also the possibility that individuals with type 2 diabetes suffer more from depression (Nichols 2003). The combination of these

metabolic alterations with a sedentary lifestyle and feelings of frustration and hopelessness related to weight loss can readily impede future weight loss efforts. The need for intensive behavioral therapy in this population is apparent. Additionally, there is a need for prospective studies examining the effect of long-term behavior strategies on weight loss in these individuals. This study compares the weight-losing effects of an appetite suppressant in obese subjects with type 2 diabetes versus an obese subject group without type 2 diabetes.

As the difference in weight lost between obese individuals with type 2 diabetes versus similar matched controls with the use of an appetite suppressant has rarely been addressed, we compared weight loss in four groups of study subjects based on the use of a long-term appetite suppressant (AXOKINE®). The first two groups of subjects participated in a phase II study (to determine safety and efficacy) whereby one treatment arm was comprised of obese subjects randomized to receive 1 μ/kg body weight of AXOKINE while obese subjects in the other treatment group received placebo. The next two groups (also participating in a phase II study) were obese or overweight subjects with type 2 diabetes mellitus whereby one treatment group received 1μ/kg body weight of AXOKINE and the other treatment arm received placebo. All four groups were studied for 12-weeks in a double-blind manner. Examining this data retrospectively, it is hypothesized that via one or more of the mechanisms described above, an obese person with type 2 diabetes will have more difficulty losing weight after 12- weeks on AXOKINE than an obese nondiabetic person taking AXOKINE for the same time period.

Specific Aims

The aims of this thesis were:

1. To determine whether AXOKINE increases weight loss compared with placebo in obese patients with and without type 2 diabetes mellitus and to assess the dose response relationship for weight loss with AXOKINE

2. To explore the short-term effects of weight loss with AXOKINE on glycemic control.

3 Methodology and Study Design

3.1 Study 1 (obese, no diabetes)

3.1.1 Subjects

Subjects were recruited through the use of advertisement and referrals at six different clinical research sites (listed in Appendix A) in the United States. The subject's eligibility was confirmed two weeks prior to starting the single-blind placebo phase of the study. All subjects gave written informed consent at the research clinic before any study procedures were performed. Key eligibility criteria were male and female subjects 18 – 70 years of age, a body mass index (the weight in kilograms divided by the square of the height in meters) of 35 to 50 kg/m^2, stable weight with no more than a 4 kg gain or loss in the previous 3 months (by history), a willingness to comply with study medication, procedures, and for men and women of childbearing potential, willingness to utilize adequate contraception and not become pregnant during the course of the study. Eligible persons were excluded if they had diabetes mellitus type I or II, history of stroke or myocardial infarction, history of coronary artery disease, angina, congestive heart failure, or arrhythmia, untreated or uncontrolled hypertension or hyperlipidemia, administration of any other weight loss drug in the three months prior to enrollment or any uncontrolled or active major systemic pulmonary, gastrointestinal, urogenital, neurological, psychiatric, or neoplastic disorder with metastatic potential.

3.1.2 Protocol

The initial phase of the study consisted of a 14-day single-blind, placebo run-in phase. Subjects were taught to self administer AXOKINE subcutaneously (Appendix C) during a 14-day placebo controlled run-in phase. Those who successfully completed this phase were randomized into the double blind treatment phase. The drug treatment phase was an 84-day, double blind, parallel group, randomized, placebo controlled dose finding study. One hundred and seventy subjects were enrolled into the study and were included into the efficacy analysis. Thirty-five subjects were randomized each to placebo, 0.3, 1.0, and 2.0 μg/kg/day of AXOKINE for 84 days. A follow-up period of six weeks led to an extended follow-up phase of one year (see timeline – figure 1).

An Investigator, Study Coordinator or Dietitian met with each subject, conducted a 24-hour food recall, and elicited information on average energy expenditure. It was recommended that the same counselor should conduct the sessions throughout the study at each site. The WHO formula (Schofield, 1985), was used to calculate the daily calorie requirements. Meal recommendations were made so that the daily food consumption was 500 calories less that the computed requirement. Meal planning sheets and calorie content for individual foods were given to each subject. Subjects were also encouraged to participate in an exercise program appropriate to their health status and lifestyle.

3.1.3 Anthropometrics and Biochemical Analysis

In addition to dietary counseling, subjects also received a waist circumference measurement at every visit and two DEXA scans during the course of the 12-week double blind study period. Additionally, two echocardiography exams and three oral glucose tolerance tests were performed in the same study period. Subjects were also

instructed to conduct heart rate self-monitoring at home, 5 days per week, throughout the study.

The extended follow-up phase was intended to collect anti-AXOKINE antibody levels and to monitor for the potential of long term adverse events. At the end of the 12-week double blind study period, return clinic visits were scheduled six weeks following the last, and at three-monthly intervals thereafter until each subject was followed for one year after the last dose of study drug administration. The study blind for individual subjects was maintained for the Investigators and subjects until the end of the extended follow-up. At these extended follow-up visits, the following procedures were conducted: measurement of anti-AXOKINE antibodies, neurological questionnaire, an adverse event inquiry, a weight measurement and dietary counseling at 6 and 12 months. Subjects received a total of six dietary lifestyle counseling sessions throughout the double blind and the follow-up period of the study.

3.1.4 Statistical Analysis

The sample size of 35 subjects per treatment group with at least 30 evaluable subjects had approximately 80% power to find at least one group different from the others with respect to weight loss. The calculation assumed a difference in weight change between the highest and lowest groups on the order of 3 kg with a common standard deviation of 3 kg.

Responder analyses (subjects with predefined decrease from Baseline) were also performed on the same data by the Cochran-Mantel-Haenszel procedure, stratified by study center. A test for trend was first performed using log (dose) as coefficients to create a one degree of freedom contrast. As with the analysis of the continuous data, if this test was significant then each active dose group was compared with placebo. All statistical tests were two-sided at a significance level of 0.05.

The primary efficacy endpoint was the change in body weight, expressed as the difference between the value measured on the day of randomization, immediately prior to the start of dosing of double-blind study drug and the value obtained at the end of the double-blind treatment period. Analysis of body weights included repeated measured analysis of variance over all study visits. Unless otherwise stated, all values shown in the tables of this paper are expressed as the mean plus or minus the standard deviation.

3.2 Study 2 (obese, diabetes)

3.2.1 Subjects

Subjects were recruited at 24 different clinical research sites in the United States (Appendix B). Eligibility criteria remained the same for the previous trial except for the following: a body mass index between 27 to 50 kg/m^2 (with a minimum weight of 80 kilograms), age 30 to 70 years, type 2 diabetes mellitus, diagnosed at least 6 months prior to screening but no more than 10 years prior to screening, current treatment of type 2 diabetes mellitus with a stable dose of sulfonylurea and/or metformin and/or a glitazone and/or an alpha-glucosidase inhibitor for at least 8 weeks prior to the first screening visit,

a fasting plasma glucose of 118 – 120 mg/dl inclusive, HbA_{1c} 7.5 – 10.5%, inclusive and a fasting plasma C-peptide greater than .26 nmol at the first screening visit. Eligible persons were excluded in this study if they had type 1 diabetes mellitus, MODY (Mature Onset Diabetes of the Young), insulin-dependent type 2 diabetes mellitus, or other unusual or rare form of diabetes mellitus, elevated blood glucose due to medical treatment or due to a concurrent medical condition other than type 2 diabetes mellitus, marked diabetic complications, including peripheral artery disease, severe autonomic or sensory neuropathy, nephropathy or proliferative retinopathy, history of diabetic coma or hypoglycemic episode requiring help, administration of an over-the-counter preparation for weight loss, gastrointestinal surgery for obesity, history or presence of malignancy within the past 5 years and uncontrolled or active major systemic pulmonary, gastrointestinal, urogenital, neurological, psychiatric, or neoplastic disorder with metastatic potential.

3.2.2 Protocol

One hundred and sixty patients with type 2 diabetes mellitus were randomized for treatment with placebo, 0.5, or 1.0 μg/kg/day of AXOKINE for 12 weeks. Following a 14-day single-blind, placebo run-in phase, the drug treatment phase was a 12 week, double-blind, randomized, placebo-controlled, parallel group paradigm. On the day of randomization, patients who successfully completed the run-in phase were randomized into the double-blind phase. Patients initially randomized to AXOKINE continued into the extension phase using the same concentration of AXOKINE that they used during the double blind phase; patients initially randomized to placebo used were provided with 1.0 μg/kg AXOKINE during the extension phase (see timeline – figure 3).

During the extension phase, the same volume of study drug is to be injected that was used at the end of the double blind phase. In the study of obese, diabetics, subjects were provided with diary cards to record each missed dose of study drug. They brought these cards with them for their clinic visit along with the drug storage box. Upon inspection of the card and the box, the number of used and unused vials was recorded on the case report form.

Dietary lifestyle counseling was scheduled periodically throughout the study for a total of 12 sessions. Either a Registered Dietitian (RD) or Certified Diabetes Educator (CDE) met with each patient and collected food recall and physical activity information. The nutrition educators used the guidelines established by the American Diabetes Association (2002) as well as the Learn program manual. Daily kilocalorie recommendations were estimated using the WHO formula (Schofield 1995).
*Daily kcal recommendation = {estimated basal metabolic rate x 1.3} - 500, where 1.3 is a factor for physical activity.

Patients were recommended a nutritionally balanced, mildly hypocaloric weight loss diet (30% of calories as fat, 50% as carbohydrate and 20% as protein, with a maximum of 300 mg/day as cholesterol.

3.2.3 Anthropometric and Biochemical Analysis

The following procedures were performed at the screening visit: physical exam, medical history, BMI calculation, chest x-ray, electrocardiogram, serum TSH, fasting glucose, C-peptide and HbA_1c. The second study visit signified the beginning of the placebo run-in period as well as the initiation of lifestyle counseling. Patients who had a change in their anti-diabetic medication(s) during the screening or run-in phase were not

randomized. Also, patients needed to have a fasting plasma glucose of 118 to 220 mg/dL, inclusive at the second study visit (the beginning of the run-in phase) to continue in the study. In addition, patients who experienced symptomatic hyperglycemia or symptomatic hypoglycemia during the screening or run-in phase were not randomized. Vital signs, fingerstick blood glucose, and weight were taken at each visit while fibrinogen, fasting insulin and C-reactive protein were measured at every other visit

Patients were instructed to self-monitor their blood glucose levels at home and to record seven values per week on a worksheet provided. Each week, at least 3 values in the fasted state were taken with one morning fasting glucose on the weekend (Saturday or Sunday).

3.2.4 Statistical Analysis

The sample size of 60 patients per treatment group (ITT) has approximately 80% power at the 5% level of significance to find at least one group different from the others with respect to weight loss. This sample size will also allow at least 80% power to detect the same overall differences between treatment groups pooling both strata. Randomization of patients to treatment groups was performed just prior to the double blind treatment phase. Because the proportion of subjects requiring antidiabetic medication dosage reduction was likely to be greatest in patients taking sulfonylureas compared with the other oral diabetes medications allowed in the study, randomization was stratified by use of a sulfonylurea at the time of randomization.

The hypothesis underlying the primary endpoint is that one or both doses of AXOKINE will cause a reduction in body weight as compared to placebo treatment. Analysis of body weights will include a repeated measures of variance over all visits.

The primary endpoint will be evaluated using analysis of covariance to compare the adjusted changes in body weight at the end of double-blind therapy as a function of treatment group assignment. If a treatment-related difference is observed, Dunnett's post-hoc test was used to compare the AXOKINE dose groups with placebo at a significant level of $p \leq 0$. Secondary efficacy analysis was regression analyses of change from baseline fasting plasma glucose (baseline-adjusted) against percent change in body weight for all treatment groups combined, and change from baseline HbA_1c (baseline adjusted) against percent change in body weight for all treatment groups combined. Unless otherwise stated, all values shown in the tables of this paper are expressed as the mean plus or minus the standard deviation.

4 Results

4.1 Demographic/Anthropometric data

4.1.1 Study 1 – Obese, no diabetes

Table 1 depicts the demographic/anthropometric data for subjects in study 1. One hundred, ninety-six subjects were enrolled in the single blind placebo run-in phase and 179 subjects were randomized to one of the four treatment arms (placebo, 0.3µg/kg, 1.0 µg/kg, 2.0µg/kg). Eighty-two percent of the subjects were female and 18% were male. Sixty-five percent of the subjects were Caucasian, 20% were black, and 14% were Hispanic. The mean BMI across treatment groups was 41.3 kg/m^2, the mean weight at baseline was 111.5 kg and the mean age was 43.5 years. One hundred and seventy subjects were considered evaluable and included in the efficacy analysis.

Subject compliance with taking study drug was generally good, according to the diary cards and counts of returned used vials. There were occasional situations such as family emergencies or subjects being called out of town unexpectedly when two or three doses were missed within a two-week period. Compliance with study procedures and sample collections was very good at all study sites, with few missing data points. Specific numbers for missing vials or missed doses are not available.

4.1.2 Study 2 – Obese, diabetes

In the second study, 466 subjects were screened. Out of these, 294 subjects did not meet entry criteria. One hundred seventy two subjects entered the single blind run-in phase. One hundred and fifty eight subjects were randomized and had at least one post-baseline visit. One hundred and forty two subjects completed the study.

As depicted in table 2, the three treatment groups were well matched for demographics and baseline characteristics. Overall, the mean age was 50.8 years, 61% of patients were female, and 70.3% were caucasian. The mean weight was 106 kg, and the mean BMI was 37.4 kg/m^2. The patterns of past medical history were similar across treatment groups. The proportion of subjects with diagnosed hypertension and/or dyslipidemia was also balanced across the treatment groups. Over half the patients in this study (55%) were either hypertensive or dyslipidemic. The number of doses missed per patient ranged from 1 to 25 in the AXOKINE 1.0 μg/kg group, 1 to 19 in the AXOKINE 0.5 μg/kg group, and 1 to 25 in the placebo group.

4.2 Weight Loss

4.2.1 Weight loss in study 1

Figure 2 illustrates weight loss for nondiabetic subjects at the end of the 12-week study period. The intent to treat (ITT) population consists of those subjects who did not complete the study but were included in the efficacy analysis and followed for safety. Subjects in all active dose groups lost weight. Subjects who received placebo gained 0.6 kg and 0.3 kg (completers and intend to treat subjects, respectively). Those who received AXOKINE at the 1.0 μg/kg dose, experienced the greatest amount of weight

loss compared with baseline (mean losses of 3.4 $\pm$ 2.5 kg and 4.02 $\pm$ 3.18 kg intend to treat and completer population).

4.2.2 Weight loss in study 2

Weight loss outcomes were similar in the second study. In figure 4 subjects in the ITT (intend to treat) group lost a total of 2.97 $\pm$ 2.62 kg of body weight after 12 weeks compared with baseline, which was significantly more ($p < 0.005$) than that lost by the placebo group (1.16 $\pm$ 2.84).

4.2.3 Comparison of weight loss between the two study groups

The graph in figure 7 compares the ITT group from the obese, diabetic group in study 2 to all completers in study 1. Weight loss per visit data was not available for the ITT subjects in study. In both subject groups weight loss began at the beginning of the run-in phase (week 2) and continued until week 8. It is at this point however, nondiabetic subjects (study 1) continue to lose weight while weight loss plateaus for subjects with type 2 diabetes (study 2). Subjects in study 1 lose another 1.2 kilogram in body weight in the 4 weeks leading up to 12 weeks while those in study 2 lose only 0.11 kilogram in the same time frame. Body composition was measured in study 1 participants. Mean measures of body composition in subjects with BMI less than 40 kg/m^2, as measured by DEXA (Table 11) showed a preferential loss of fat over lean body mass at the targeted dose of 1.0 μg/kg. No changes were observed in fasting or two-hour post prandial blood glucose or insulin levels, in HbA_{1c} levels or in any of the serum lipid measurements.

4.2.4 Parameters of diabetes in study 2

Several biologic parameters were assessed to measure diabetes impact/status in this population. The data in Table 4 indicates that there was no statistically significant reduction in HbA_{1c} (-.34 $\pm$ .89 %), fasting plasma glucose (-15.5 $\pm$ 37.31 mg/dL) and fasting plasma insulin (-2.32 $\pm$ 7.61 mU/mL) in the AXOKINE (1.0 μg/kg) treated patients versus placebo. The goal of medical therapy for treating type 2 diabetes is to aim for a 1.5 to 2.0 percentage point reduction in HbA_{1c} and a 60 to 70 mg/dL drop in fasting blood glucose. As reflected in Table 4 subjects in both AXOKINE dose groups were not able to achieve these objectives.

Table 5 (figure 5) shows the amounts of oral anti-diabetic medication taken by the subjects with type 2 diabetes. Metformin (83%), sulfonylureas (66%) and thiazolidinediones (23%) were the most popular anti-diabetic agents taken subjects in study 2 (AXOKINE 1.0 μg/kg). This is representative of treatment strategies for patients with type 2 diabetes mellitus, whereby the first line of treatment is typically a sulfonylurea as a monotherapy, frequently followed by a combination therapy of metformin, TZD's and sulfonylureas (DeFronzo 1999). Metformin is becoming more popular since it doesn't cause hypoglycemia when used alone and it provides good blood sugar control in obese patients with type 2 diabetes (UKPDS 1998). Table 6 shows that more than half (60.4%) of the subjects in the 1.0 μg/kg AXOKINE treated group were taking combination therapy to treat their diabetes.

Figure 6 shows that weight loss in the 1.0μg/kg AXOKINE treatment group weight loss is similar for all groups separated by type of medication. Thiazolidinediones (TZD's) and sulfonylureas are known to cause weight gain in patients with diabetes.

5 Discussion

Although the specific mechanisms are unclear, there is evidence that individuals with type 2 diabetes are less successful in their weight loss efforts than weight-matched cohorts (Guare 1995). In this study, there were differences between the two subject groups that could have led to meaningful differences in weight loss. First, subjects with type 2 diabetes tended to be slightly older (by an average of 7 years), 5% leaner and were comprised of 25% more males in the same dosage group (1.0 μg/kg) than the nondiabetic subjects. A higher BMI in the nondiabetic group could have increased the mean weight loss more so than the mean weight lost by the group with diabetes. Also, since the nondiabetic subjects were also younger and less likely to suffer from co-morbidities such as vision, foot and cardiovascular problems they could have been predisposed to lose weight more successfully the diabetic subjects. Ultimately however, the mean weight loss between the two groups was similar.

One key component in study 2 was that the subjects were required to be on a stable dose of an oral hypoglycemic agent (sulfonylureas, alphaglucosidase inhibitors, metformin and thiazolidinediones). Insulin was excluded. As new standards of care set increasingly lower HbA_{1c} levels, more medications that enhance anabolism and weight gain are prescribed. Typically, about 75 percent of the patients with type 2 diabetes treated initially with a sulfonylurea will not achieve the desired goal of maintaining fasting blood sugar of <126 mg/dl and will require a second oral agent (or bedtime insulin)(DeFronzo 1999). Thiazolidinediones (TZD's) and sulfonylureas have been shown to increase weight in diabetic populations. In this study, weight loss outcomes for

the diabetic subjects treated with metformin, sulfonylureas and TZD's were similar to the weight loss outcomes of the nondiabetic obese subjects in study 1.

In terms of diet, subjects in study 1 were provided a low-fat, healthy plan (WHO formula – 500 kcals/day) while participants in study 2 were given counseling in accordance with the LEARN® program. The LEARN program, which stands for lifestyles, exercise, attitudes, relationships, and nutrition is a comprehensive manual incorporating all of these parameters with LEARN® centered counseling. The frequency of counseling in study 2 occurred every 1 to 2 weeks for a total of 9 meetings, while subjects in study 1 had 4 counseling sessions (screening, day 1, day 28 and day 56). Even though the nondiabetic subjects achieved significant weight loss (compared with placebo) by the end of the study period, they may have faired better with additional behavioral modification therapy. The diabetic subjects may have also lost more weight with increased behavioral modification therapy and an exercise regimen tailored to their specific abilities.

It is hard to know whether a longer study period would have enhanced weight loss in either or both study groups. Although Registered Dietitians provided a comprehensive lifestyle approach to the subjects in study 2, the weight loss outcomes were disappointing, with respondents only losing 2.8 percent of their baseline weight (AX 1.0µg /kg BW). Unfortunately, a comprehensive approach does not always guarantee that all of a patient's needs for weight loss will be met. In fact, disappointing results like ours are apparent even in outstanding medical centers with exceptional clinicians and facilities. One such case is the Redmon et al., study whereby overweight or obese individuals with type 2 diabetes were randomly assigned to either a combination therapy weight loss

program for 2 years or a standard therapy weight loss program for one year followed by a combination therapy weight loss program in the 2nd year. After 2 years, the combination therapy group had a mean weight loss of 4.6 ± 1.2 kg (Redmon 2005). One would have expected a more auspicious weight loss, especially since the combination therapy component of this program offered meal replacement products, periods of low-calorie intake, sibutramine, and nutritional counseling (Redmon 2005). These results and the outcomes of other well controlled studies using multiple approaches underscore the need for a more thorough lifestyle approach for those with type 2 diabetes (Brown 1996, Wing 1993).

In stratifying weight loss between subject groups with diabetes, the identification of their stage of disease may prove to be advantageous. Although data on length of time with type 2 diabetes was not captured in study 2, there is evidence that diabetes disease stage is another factor that could obstruct weight loss efforts. A study by Khan et al., observed a significant linear trend ($p < .001$) for decreasing weight loss with stage of diabetes. Patients with the highest levels of HbA_{1c} had the least change in BMI. Also, patients without diabetes had a greater mean reduction in BMI than group with diabetes (Khan 2000.) Typically, the A1C goal for patients is a measurement of < 7% (ADA 2006). Data from the UKPDS indicate that HbA_{1c} tends to increase by 0.05 – 0.15% per year in obese individuals with type 2 diabetes (UKPDS 1998, Knowler 2002). The mean HbA_{1c} in study 2 (AXOKINE 1.0 μ/kg) was 8.17 %. The higher measurements of HbA_{1c} in study 2 indicate that the disease progression of the study subjects was possibly worsening.

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Other caveats in the methodology of these studies were that food intake and exercise was not measured. The two populations could have also been better matched in terms of age, BMI, weight and sex. As mentioned previously, subjects in study 1 could have had increased mean weight loss secondary to a higher baseline BMI. Successful weight loss may have been attenuated for subjects in study 2 by the presence of co-morbidities (hypertension, dyslipidemia), combination oral hypoglycemic therapy and disease progression.

The presence of depression could affect one's ability to lose weight. Individuals with type 2 diabetes have been shown to suffer from a higher prevalence of depression than their nondiabetic cohorts. Nichols et al. compared the prevalence of diagnosed depression in 16,180 HMO members in 1999, diagnosed with type 2 diabetes and compared them to the same number of members without diabetes (matched for age and sex). After adjusting the prevalence estimates for cardiovascular disease, age, sex and body weight, depression was found to be more common in the type 2 diabetics than the control subjects (17.9% vs. 11.2%, $P< 0.001$). Also, body weight was found to be a much stronger predictor of depression than the presence of diabetes (Nichols 2003). It would have been interesting to see if there was measurable dysphoria in both subject groups for this study. Extrapolating these results to study's participants in study 2 could mean that not only were they more likely to suffer from depression because of their body weight, but the presence of type 2 diabetes may have further exalted their sense of dysphoria.

Based on the methodology utilized in this study, the results of studies 1 and 2 disprove the hypothesis that obese subjects with diabetes have more difficulty losing weight than obese subjects without diabetes.

5.1 Future Directions

Even though there is world-wide consensus among health practitioners that a healthy diet combined with an exercise program is the best way for overweight and obese people to lose weight, obesity and diabetes are still spiraling towards staggering figures. People generally know what they have to do to lose weight, they are just too addicted to their lifestyles, pattern of food consumption and alone in their battles to make the changes they need to. Once a state of obesity has been determined, the path for diabetes is paved with the reduced potential for weight loss in the future. Pi-Sunyer has stated in a recent article that those with type 2 diabetes are likely to be frustrated as they have more difficulty losing weight not only because of their altered metabolism, but also because they suffer from feelings of helpless, powerlessness, complications from diabetes and effects of antidiabetes medication (Pi-Sunyer 2005).

Research now calls for a more intensive approach to helping those with type 2 diabetes change their lifestyle habits, citing the need for several techniques to be used in the office setting (NIH 2000, Poston 2000, Wing 2001, Wadden 2000). These techniques would involve the initial identification of problem behavior(s), agreement of specific, realistic goals, and incorporating strategies such as self-monitoring (daily records of food intake and physical activity), stimulus control (avoiding triggers that prompt eating), and problem solving (identifying barriers and ways to overcome them). It is also advised to have frequent patient-provider contact (e.g., weekly or biweekly) as this associated with

better long-term weight maintenance (Anderson 1999). Although these methods may prove useful in helping an overweight person with type 2 diabetes and/or an obese person lose weight, the real challenge however, lies in preventing obesity.

As children are raised with more junk and fast food advertising then ever, they are simultaneously living a wired lifestyle – spending much less time being active. Adults are no better, more often than not, driving for most or all errands and also spending more time on the computer or in front of the TV. Combining these lifestyle habits with an overabundance of cheap, highly palatable food is toxic. People who are obese and/or are suffering from type 2 diabetes need more help from their community and health care practitioners. Not only should the intensive approach strategies listed above be incorporated into counseling, stronger community health programs aimed at increasing physical activity and better nutrition are needed.

In addition to elucidating the mechanisms behind the metabolic alterations seen in those with type 2 diabetes (higher free fatty acid flux, higher energy expenditure, lower GLP-1 levels), researchers should use small focused studies to identify the factors (environmental/lifestyle) that obstruct weight loss efforts. This would allow for development of strategies to change those influences (i.e. limit television, eating out less often, etc.), followed by the evaluation of strategies that were implemented. Methods used to maintain weight loss in weight loss programs should be analyzed and clinical research studies designed to focus on weight loss with sizeable populations should also focus more on long term weight loss maintenance (Wing 2001). Future studies should strive to gather comparative data on weight loss between obese diabetic and nondiabetic groups to realize the factors that may cause a discrepancy. Additionally, more controlled

prospective studies are needed to examine strategies for long-term weight loss in people with type 2 diabetes (Redmon 2005). The ongoing LookAHEAD study is an 11-year prospective controlled trial sponsored by the NIH. Its objectives are to assess whether long-term weight loss is achievable and beneficial in overweight individuals with type 2 diabetes (www.niddk.nih.gov/patient/show/lookahead.htm).

An obese or overweight person with diabetes will thus need not only a combination therapy approach, but a team approach. A team could consist of other people with diabetes struggling to lose weight (teammates), coaches (in the form of community health teachers of exercise, nutrition and behavioral counseling), and most important, cheerleaders (family, friends), providing unconditional support and encouragement. Ultimately, researchers need to identify the mechanisms that hold people back from successful weight loss and community leaders need to organize and fund the programs that will help them. These activities will be fundamental for obesity and diabetes prevention as well as treatment. Preventing the diabetic state would save millions in health care spending as well as augmenting the health and lifespan of those at risk. Currently however, as millions more become diabetic, the need for a community-based, intensive approach is urgent.

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Appendix A. Clinical Research Sites in Study 1

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Sherwyn L. Schwartz, M.D.	Diabetes and Glandular Disease Clinic, P.A. 8042 Wurzbach, Suite 420 San Antonio, TX 78229-3894
Stuart Weiss, M.D.	The San Diego Endocrine and Medicine Clinic 5920 Friar's Road, Suite 208 San Diego, CA 92108
George Bray, M.D.	Pennington Biomedical Research Center 6400 Perkin's Road Baton Rouge, LA 70808
Harris Mcllwain, M.D.	Tampa Medical Group Research 4700 N. Habana Avenue, Suite 303 Tampa, FL 33614
Thomas Littlejohn III, M.D.	Piedmont Medical Research Associates 1901 S. Hawthorne Road, Suite 306 Winston-Salem, NC 27103

Appendix B. Clinical Research Sites in Study 2

Andrew Ahmann, MD	Radiant Research 5331 SW Macadam Ave. Portland, OR 97201
Harold Bays, MD	L-MARC Research Center 3288 Illinois Ave. Louisville, KY 40213
Gordon Connor, MD	Radiant Research 516 Brookwood Blvd. Birmingham, AL 35209
Priscilla Hollander, MD	Baylor University Medical Center Wadley Tower 3600 Gaston Ave. Dallas, TX 75246
David Morin, MD	Tricities Medical Research 1958 W. State St. Bristol, TN 37620
Michael Noss, MD	Radiant Research 7720 Montgomery Road Cincinnati, OH 45236
Bryan Pogue, MD	Radiant Research 6565 West Emerald Boise, ID 83704
Sherwyn Schwartz, MD	Diabetes and Glandular Diseases Clinic, PA 5107 Medical Drive San Antonio, TX 78229
Diane Smith, MD	CSRA Partners in Health, Inc. 1220 Augusta Way Pkwy Augusta, GA 30909
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Daniel Thompson, MD	Advanced Healthcare, S.C. Milwaukee Medical Clinic 3003 W. Good Hope Rd. Milwaukee, WI 53209
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Appendix C. Methodology

C.1 Study Drug Administration: Study 1 and Study 2

Patients were instructed to self-administer study drug by subcutaneous injection once daily. Patients were instructed on aseptic technique for self-injections and allowed to practice until they reached a level of competency acceptable to the study staff. Patients must have demonstrated competency at drawing into the syringe the intended volume of injectate, and competency at injecting into the abdomen and thighs, and agreed to rotate injections during the study among most of the following anatomical sites:

Site 1: Left middle abdomen region (at least 2 inches away from the umbilicus)

Site 2: Right middle abdomen region (at least 2 inches away from the umbilicus)

Site 3: Left lower abdomen region (at least 2 inches away from the umbilicus)

Site 4: Right lower abdomen region (at least 2 inches away from the umbilicus)

Site 5: Lateral aspect of the left middle thigh

Site 6: Lateral aspect of the right middle thigh

Site 7: Lateral aspect of the left lower thigh (at least 2 inches above the upper edge of the patella)

Site 8: Lateral aspect of the right lower thigh (at least 2 inches above the upper edge of the patella)

Site 9: Medial aspect of the left middle thigh

Site 10: Medial aspect of the right middle thigh

Site 11: Medial aspect of the left lower thigh (at least 2 inches above the upper edge of the patella)

Site 12: Medial aspect of the right lower thigh (at least 2 inches above the upper edge of the patella)

The importance of rotating injections among all sites was emphasized to the patients to minimize the risk of a site reaction or infection.

C.2 Study removal: Study 1 (obese, no diabetes)

Subjects were removed from the study if any one or more of the following events occurred: noncompliance with protocol by the subject, intolerable adverse event, decision by the Investigator or the sponsor that termination was in the subject's best medical interest or administrative decision for a reason other than that of an adverse event, request for withdrawal by the subject for reasons other than an intolerable adverse event or loss to follow up.

C.3 Change in body composition: Study 1

Whole body dual energy x-ray absorption (DEXA) was performed at Visits 4 and 12 for all subjects who did not exceed the weight limit imposed by the DEXA machine. Subjects underwent the test locally, there was no cross center calibration of the DEXA instruments, and each study site applied its own quality assurance program.

Percent loss of fat was calculated by dividing the DEXA measured change in fat (kg) by the DEXA measured total weight change (kg).

C.4 Subject recruitment: Study 2 (obese, diabetes)

All patients signed an informed consent prior to the conduct of any study-related procedures. Patients were told that they would receive placebo at some time during the course of the study and they were made aware that they could not use any other drugs for weight loss (prescription/over-the-counter/herbal products), undergo weight loss surgery, liposuction, breast reduction or augmentation, and could not become pregnant (or impregnate their partners).

Appendix D. Result Details

D.1 Protocol Deviations: Study 1

Multiple, minor protocol deviations were documented over the course of the study. The most frequently occurring deviation concerned subjects attending clinic visits outside of the designated window. As the study ran from March until October, 2000, this was compounded by summer vacation schedules. Although the rule was that subjects could miss no more than three doses per week in order to remain in the study, some subjects did miss more than three doses each week.

One deviation that affected all study subjects that had begun dosing involved the drug recall and replacement that occurred in May, 2000. This resulted in a gap in dosing of one to seven days, dependent on how soon subjects could return to the clinic for their replacement packer. No subjects were discontinued related to this interruption of dosing.

Four subjects received medications that were in violation of the protocol. These medications included tamoxifen (for breast cancer), and paroxetine hydrochloride (for depression).

D.2 Change in body composition: Study 1

The number of subjects who had a baseline and follow-up DEXA assessment was relatively small in each dose group. Since the entry criteria for this study specified a BMI range of 35-50 kg/m^2, many of the subjects in this trial were too heavy to fit on the instrument's table. The percent error increased significantly for those with a BMI higher than 40 kg/m^2. Furthermore, it appeared that the signal to noise ratio was unfavorable in

that the standard deviations were large when compared with the means. The correlation between the DEXA measured weight changes and the weight changes measured on the scales vary between dose groups. Consequently, the calculated percent fat loss was highly variable and associated with a large confidence interval. Due to these limitations, the DEXA analysis was then conducted only on those subjects with BMI less than 40 kg/m^2; these data may be found in Table 10.

D.3 Study deviations: Study 2

There were a total of 178 documented protocol deviations noted with waivers granted. The most frequently occurring deviations were out of window visits (44 reports), fasting plasma glucose out of range (35 reports), and HbA_{1c} out of range at Screening (32 reports). Other deviations included out of range BMIs, history of diabetes for more than 10 years, exclusionary laboratory values, and exclusionary concomitant medications.

D.4 Study withdrawal information: Study 2

Eighteen of the 160 patients (11.3%) withdrew from the study prematurely during the double blind phase. During the open label phase, a total of six patients (3.8%) discontinued prematurely: two patients withdrew consent, two patients reported an intolerable adverse event, one patient requested withdrawal, and one withdrew secondary to Investigator decision. The incidence and reasons for early withdrawal were comparable across treatment groups.

D.5 Adverse Events: Study 2

During the single blind, run-in phase of the study, 53 of 160 patients (33.1%) reported at least one adverse event; during the double blind phase, 118 of the 160 patients (73.8%) reported at lest one adverse event. The most frequently reported treatment emergent adverse events were injection site reactions, nasopharyngitis, upper respiratory infection, hypoglycemia, and nausea.

D.6 Adverse Events: Study 1

The most commonly reported drug-related adverse events in study 1 were injection site reactions, nausea, and cough. The incidence of these adverse events did appear to be dose dependent. The injection site reactions were described as non-indurated, erythematous areas measuring up to 1x4 cm with ecchymosis and occasional pruritis. These areas appeared about a day after an injection and resolved spontaneously within several days. Nausea was reported in 9 of 38 subjects receiving AXOKINE 1.0 µg/kg daily and 11 of 33 subjects receiving AXOKINE 2.0 µg/kg daily, with no reports of severe nausea; 6 subjects required prescription or over-the counter (OTC) medication for nausea. Cough was reported in 7 of 108 subjects (6.5%) in the AXOKINE dose groups of 1.0 µg/kg or less daily, whereas cough was reported in 14 of 33 subjects (42%) treated with AXOKINE 2.0 µg/kg daily; 9 subjects required treatment for cough with prescription or OTC medication.

Tables

Table 1: Baseline characteristics for study 1

	Placebo	0.3 µg/kg	1.0 µg/kg	2.0 µg/kg
Randomized	**34**	**33**	**38**	**35**
Age (yrs)	**41.7 ± 11.2**	**42.8 ± 10.7**	**43.5 ± 10.1**	**41.1 ± 9.7**
Female (%)	**26 (81.3)**	**26 (81.3)**	**32 (84.2)**	**26 (78.8)**
Male (%)	**6 (18.8)**	**6 (18.8)**	**6 (15.8)**	**7 (21.2)**
Ethnicity(cauc/AA/other)	**17/11/4**	**20/7/5**	**26/7/5**	**23/4/6**
Weight (kg)	**111.4 ± 13.9**	**116.1 ± 17.5**	**111.5 ± 15.4**	**117.4 ± 17.8**
BMI ($kg/m^{2)}$	**40.5 ± 3.4**	**41.7 ± 4.1**	**40.6 ± 3.9**	**42.2 ± 4.3**
Fasting plasma glucose (mg/dL)	**94.6 ± 12.5**	**96.0 ± 9.5**	**101.8 ± 16.6**	**99.3 ± 12.6**

All values are shown as the mean ± standard deviation (SD)

Table 2: Baseline characteristics for study 2

	Placebo	0.5 µg/kg	1.0 µg/kg
Randomized	**53**	**53**	**54**
Age (yrs)	**51.3 ± 8.4**	**50.7 ± 8.0**	**50.5 ± 8.9**
Female (%)	**32 (60.4)**	**34 (65.4)**	**31 (58.5)**
Male (%)	**21 (39.6)**	**18 (34.6)**	**22 (41.5)**
Ethnicity (cauc/AA/other)	**66/15/19**	**77/8/15**	**68/21/11**
Weight (kg)	**105.1 ± 19.9**	**106.7 ± 20.6**	**106.2 ± 16.7**
BMI ($kg/m^{2)}$	**37.1 ± 5.4**	**37.8 ± 4.8**	**37.2 ± 5.3**
Fasting plasma glucose (mg/dL)	**163 ± 34.8**	**171 ± 37.5**	**174 ± 36.7**

All values are shown as the mean ± standard deviation (SD)

Table 3: Change in body weight – baseline to week 12

Study 1 (AX) (ITT)	**PL (N=31)**	**0.3 µg/kg (N=31)**	**1.0 µg/kg (N=37)**	**2.0 µg/kg (N=33)**
Change from baseline (kg)	+0.27 ± 2.78	-1.09 ± 2.8	-3.4 ± 2.5	-2.61± 2.9
p-value vs. placebo*		0.038	<0.0001	<0.0001
Study 2 (AX) (ITT)	**PL (N=53)**	**0.5 µg/kg (N=52)**	**1.0 µg/kg (N=53)**	
Change from baseline (kg)	-1.16 ± 2.84	-2.36 ±3.63	-2.97 ± 2.62	
p-value vs. placebo*		0.97	0.005	

All values are shown as the mean ± standard deviation (SD)

*** Dunnett's post-hoc test was used to compare treatment to placebo**

Table 4: Mean change from baseline to week 12 in metabolic parameters – study 2

	AXOKINE 1.0 µg/kg N = 53	**AXOKINE 0.5 µg/kg N = 52**	**Placebo N = 53**	**p-value**
HbA_{1c} (%)	-0.34 ± 0.89	-0.02 ± .93	-0.22 ± 1.11	0.31
Fasting plasma glucose (mg/dL)	-15.5 ± 37.31	-12.5 ± 34.88	-12.4 ± 37.11	0.87
Fasting plasma insulin (mU/mL)	-2.32 ± 7.61	-3.82 ± 9.70	0.55 ± 6.48	0.06

All values are shown as the mean ± standard deviation (SD)

Table 5: Baseline demographics/characteristics for percent of individuals using anti-diabetic medications in Study 2

AXOKINE	**AX 1.0 μg/kg**	**AX 0.5 μg/kg**	**Placebo**
Alphaglucosidase Inhibitors	0%	0%	2%
Metformin	83%	75%	91%
Insulin	0%	0%	0%
Meglitinides	2%	0%	0%
Sulfonylureas	66%	67%	68%
Thiazolidinediones	23%	35%	21%

Table 6: Baseline demographics/characteristics antidiabetic medications- by medication

	AXOKINE		Placebo
	1.0 µg/kg	0.5 µg/kg	
Metformin Only	28.3%	19.2%	24.5%
Sulfonylureas Only	9.4%	11.5%	1.9%
Thiazolidinediones Only	1.9%	3.9%	1.9%
Multiple Medications	60.4%	65.4%	71.7%

Table 7: Disposition of patients – study 2

	AXOKINE		Placebo
	µcg/kg	µg/kg	
Patients Randomized:	54	53	53
Completed Double Blind phase:	47	47	48
Completed Open Label:	46	45	45

Table 8: Incidence of hypertension and/or dyslipidemia – study 2

		AXOKINE		Placebo N = 53
		1.0 µg/kg N = 54	0.5 µg/kg N = 53	
Hypertension N (%)	Yes	29 (54.7)	31 (59.6)	30 (56.6)
	No	24 (45.3)	21 (40.4)	23 (43.4)
Dyslipidemia N (%)	Yes	29 (54.7)	33 (63.5)	42 (79.3)
	No	24 (45.3)	19 (36.5)	11 (20.8)

Table 9: Weight change (kg) by baseline antidiabetic medication class – study 2

		AXOKINE		Placebo
		1.0 µg/kg	0.5 µg/kg	
Biguanides	Weight Change (kg)	-3.2 ± 2.65	-2.7 ± 3.75	-1.2 ± 2.74
	N	44	39	47
	p-value vs. Placebo	0.006	0.07	-
Thiazolidinediones	Weight Change (kg)	-3.4 ± 2.77	-2.4 ± 2.97	-0.2 ± 2.65
	N	12	18	11
	p-value vs. Placebo	0.016	0.134	-
Sulfonylureas	Weight Change (kg)	-3.2 ± 2.95	-2.0 ± 2.95	-0.9 ± 2.36
	N	35	35	35
	p-value vs. Placebo	0.002	0.196	-

All values are shown as the mean ± standard deviation (SD)

Table 10: Subject disposition for study 1

	Placebo	0.3 µg/kg	1.0 µg/kg	1.0 µg/kg-P	2.0 µg/kg	Totals
Screened						316
Randomized	34	33	38	39	35	179
Evaluated for safety	32	32	38	38	33	173
Evaluated for efficacy	31	31	37	38	33	170
Completed double blind	23	24	27	30	19	123

Table 11: Changes in body composition for subjects with BMI <40 kg/m^2 study 1

	N	Δ Fat (Kg)	Δ Total (Kg)	% Fat Loss
Placebo	8	-0.76 ± 2.12	0.54 ± 2.28	-141.0
0.3 µg/kg	6	-1.39 ± 2.32	-1.44 ± 4.21	97.0
1.0 µg/kg	12	-2.13 ± 2.04	-2.56 ± 2.77	83.2
1.0 µg/kg – P	15	-0.29 ± 2.04	-0.70 ± 2.09	41.0
2.0 µg/kg	6	-2.32 ± 1.70	-4.11 ± 2.85	56.0

All values are shown as the mean ± standard deviation (SD)

Figures

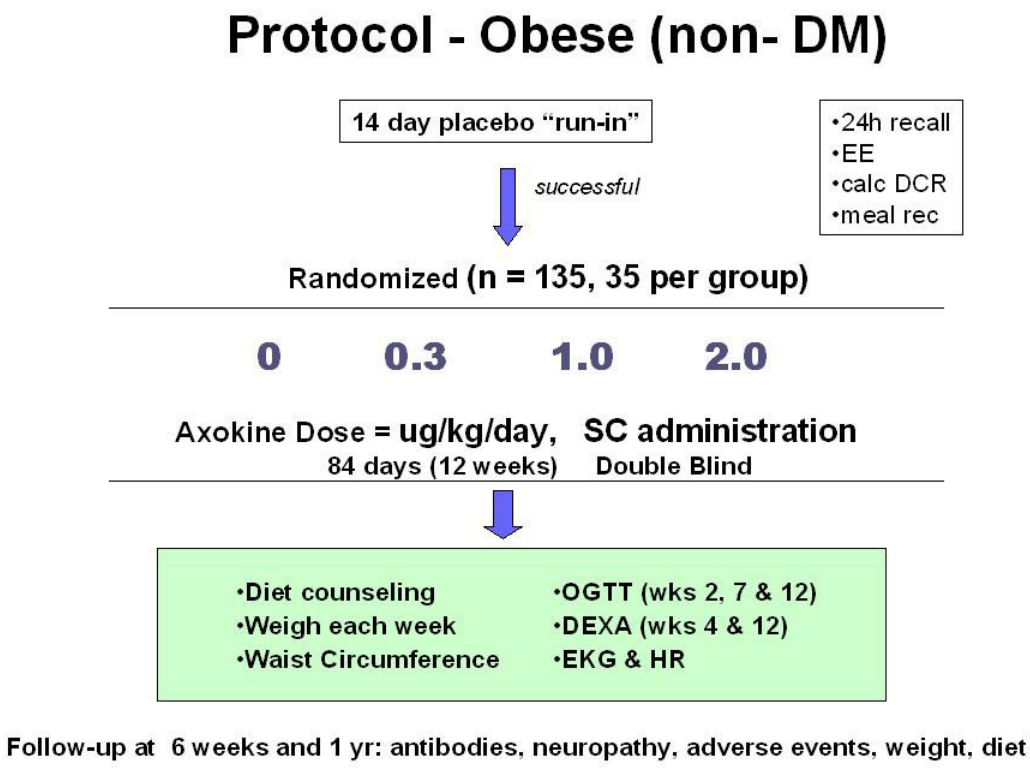

Figure 1: Timeline for study 1

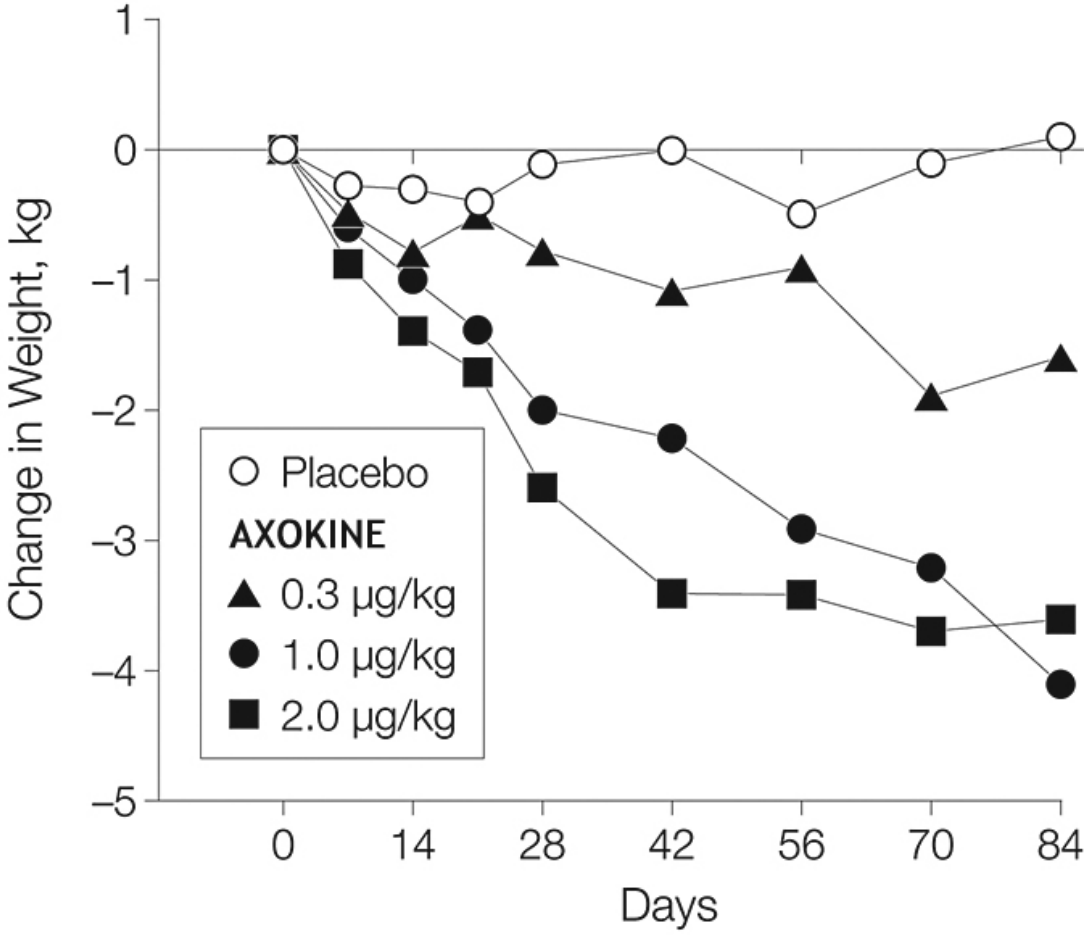

Figure 2: Mean weight loss study 1

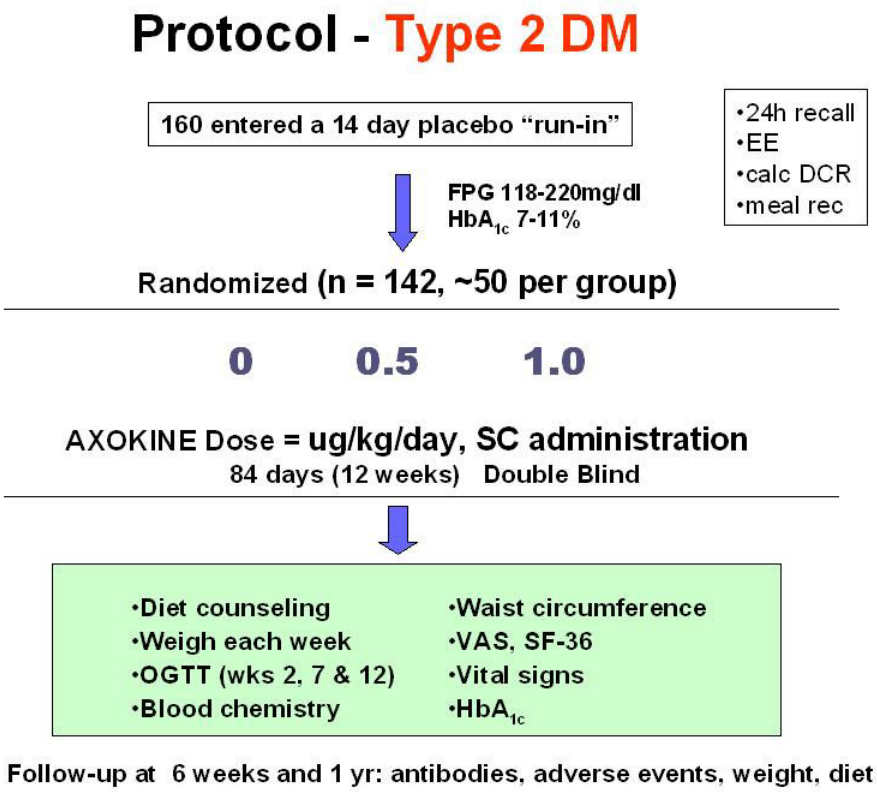

Figure 3: Timeline for Study 2

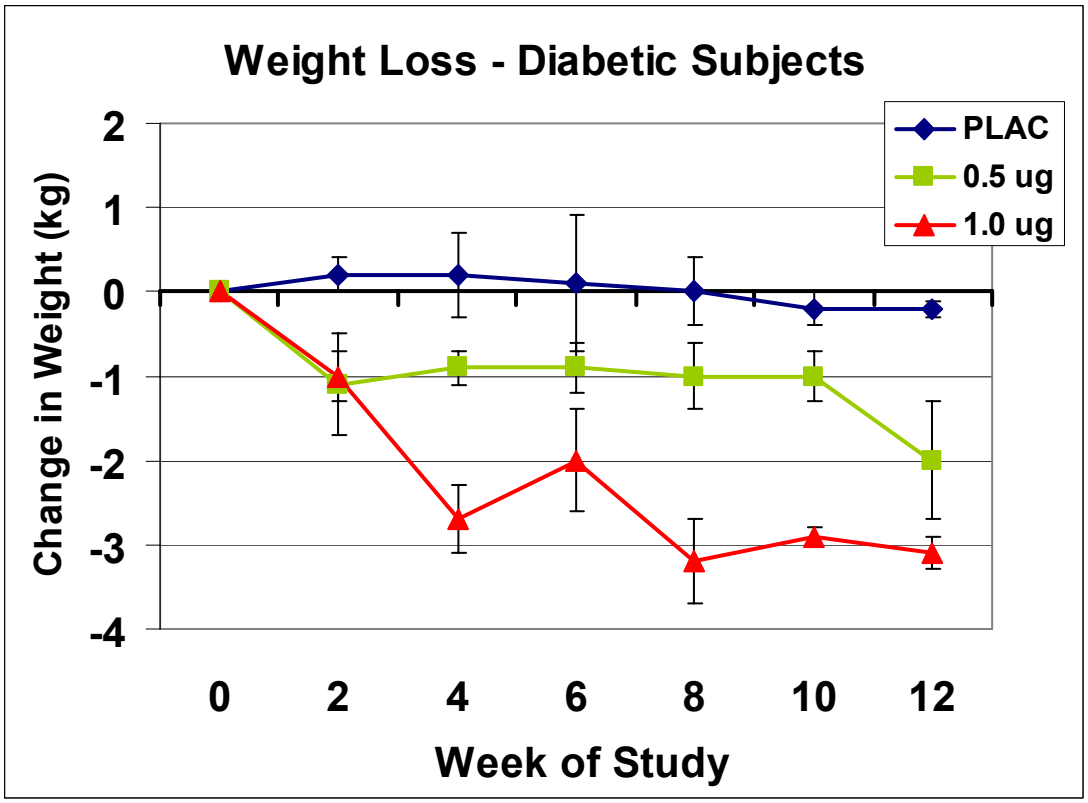

Figure 4: Mean weight loss study 2

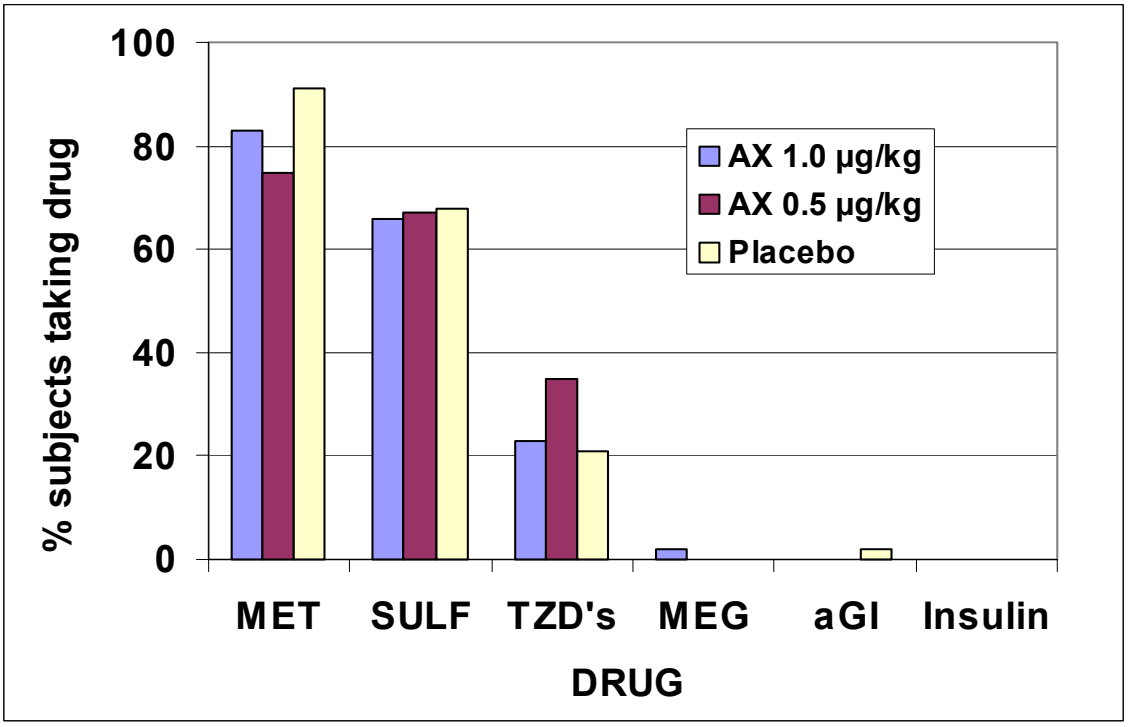

Figure 5: Similar anti-diabetic drug profiles per AXOKINE dose group – study 2

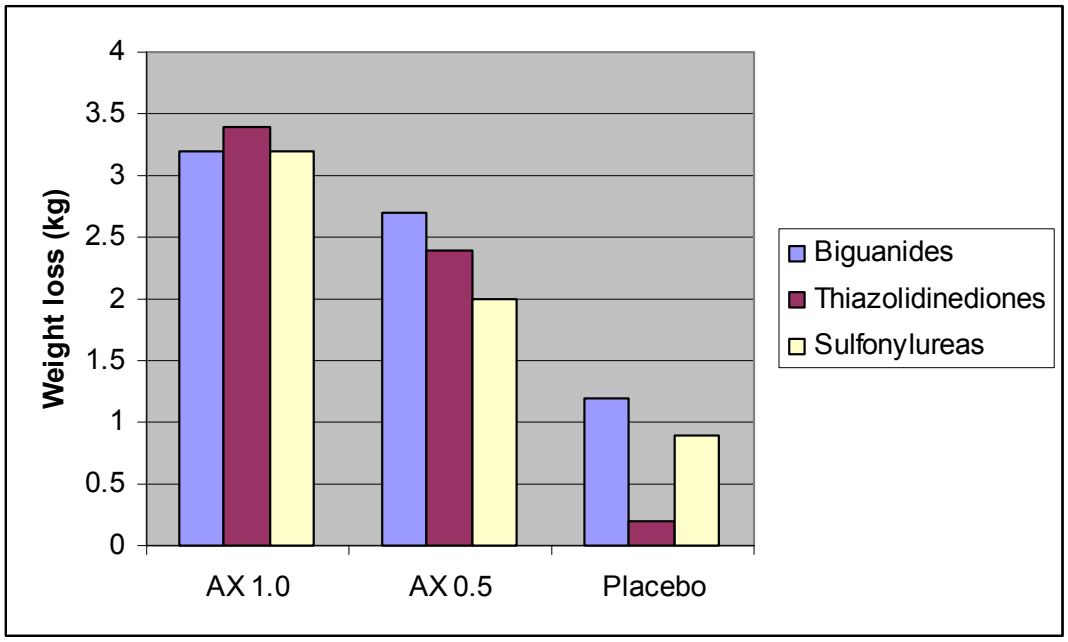

Figure 6: Weight loss at week 12 using AXOKINE stratified by anti-diabetic medication

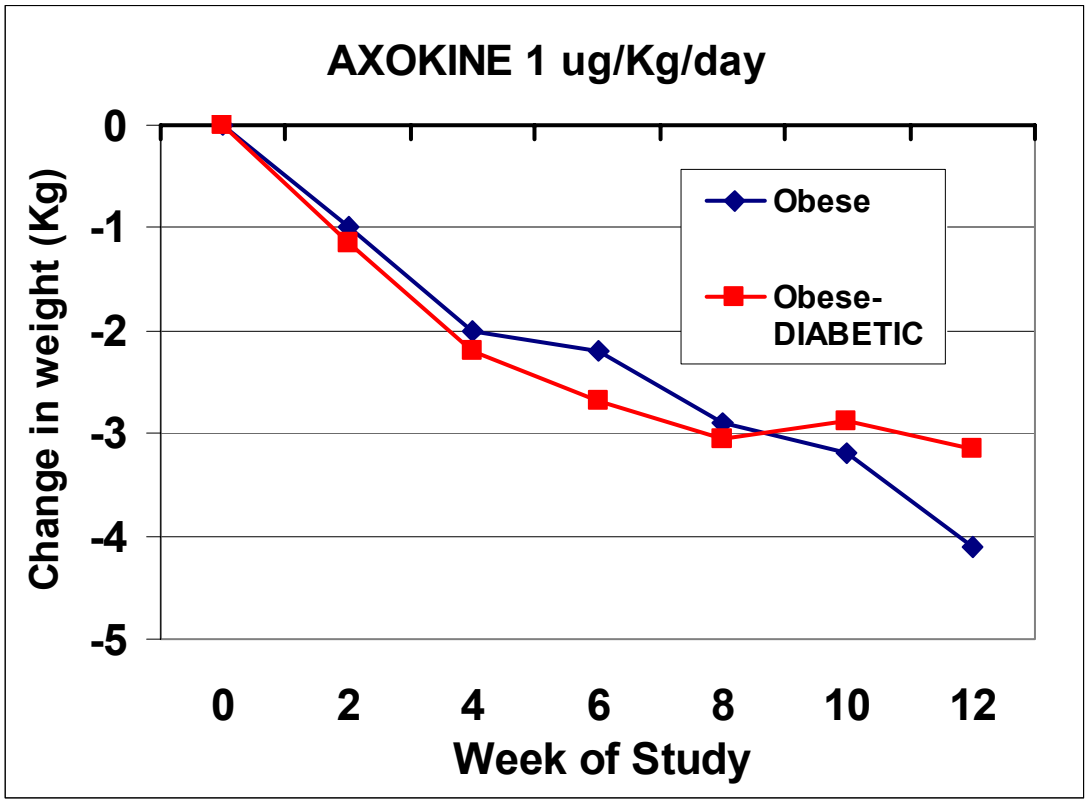

Figure 7: Comparison of weight loss in study 1 and study 2