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The Use of Exogenous ACRP30 as a Promising Amelioratory Compound Regarding Insulin Resistance and Type II Diabetes Mellitus

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THE USE OF EXOGENOUS ACRP30 AS A PROMISING AMELIORATORY COMPOUND REGARDING INSULIN RESISTANCE AND TYPE II DIABETES MELLITUS

by

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ABSTRACT

As numerous bodies of research have characterized the adipocytokine adiponectin (ACRP30) as an anti-diabetic compound, this work serves as an analysis to partly elucidate the potential usage of supplementary adiponectin as a therapeutic compound in the treatment of type II diabetes mellitus. In this work, a meta-analysis was conducted to centralize large amounts of data on adiponectin's insulin sensitizing characteristics in conjunction with like-minded studies utilizing metformin treatment as the diabetic standard of care. Upon comparison of fasting glucose, fasting insulin, and glucose tolerance test area under the curve reduction percentages, it is quite clear that ACRP30 has significant insulin enhancing properties in mouse models rivaling SOC metformin. Yet, inconsistent dosing comparisons in this analysis, the unknowns of chronic hyperadiponectinemia in humans, as well as variations in age of human diabetic patients warrant further standardized research to suggest that adiponectin supplementation can rival that of modern-day standard of care for type II diabetes mellitus.

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I would like to express my gratitude to Dr. David Flory for guiding me through this journey and his consistent support over the last eight months. In addition, I would like to thank my parents for their unwavering support.

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COMMON ABBREVIATIONS UTILIZED

ACC – Acetyl-CoA carboxylase

ACRP30 – Adiponectin

AKT – Protein Kinase B

APPL1 – adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1

BMI – Body Mass Index

CDC – Center for Disease Control

EGP – Endogenous Glucose Production

FAS – Fatty Acid Synthase

FFA – Free fatty acids

G6PASE – Glucose-6-phosphatase

GPCR – G-protein coupled receptor

HFD – high fat diet

HMW – high molecular weight

IDDM – Insulin Dependent Diabetes Mellitus

IKK-β – inhibitor of nuclear factor kappa-B kinase subunit beta

IL-6 – Interleukin 6

IRS – Insulin Receptor Substrate

MAPK – Mitogen Activate Protein Kinase

NF-kβ – NF kappa beta

NIDDM – Non-insulin dependent diabetes mellitus

OGTT – Oral Glucose Tolerance Test

PEPCK- Phosphoenolpyruvate carboxykinase

PKC – Protein Kinase C

PPAR- α – Peroxisome proliferator-activated receptor alpha

RBP4 – Retinol Binding Protein 4

T2DM – Type II diabetes mellitus

TNFα – Tumor Necrosis Factor alpha

TZDs – Thiazolidinediones

INTRODUCTION

Diabetes Mellitus is a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Among this group are autoimmune type 1 diabetes, monogenic diabetes, type 2 diabetes (NIDDM), latent autoimmune diabetes in adults, and gestational diabetes (Kharroubi, A. T., & Darwish, H. M., 2015). According to the latest National Diabetes Statistics Report by the Center for Disease Control (CDC), diabetes has been quantified in an alarming 10.5% of the US population (CDC, 2020). Type 2 diabetes is the most prevalent classification of diabetes among adults worldwide (Kharroubi, A. T., & Darwish, H. M., 2015). With the upward trend witnessed in the CDC report, potential ameliorations should be at the forefront of research. Type 2 diabetes typically arises due to a combination of a sedentary lifestyle, obesity, poor diet, smoking, significant alcohol consumption, and the supported presence of known pro-diabetic genes (Olokoba, A. B., Obateru, O. A., & Olokoba, L. B., 2012). Multiple therapeutics exist, yet the therapeutic control of glycemia can still fail. (Achari, A. E., & Jain, S. K., 2017). Many therapeutics exist such as Metformin, Glimepiride, Voglibose, Sitagliptin, thiazolidinediones, and Gliflozin category drugs (Banerjee et al. 2019). Yet, each of these possible therapeutics expect the possibility of quite negative side effects along with the potential of failed treatment (Banerjee et al. 2019).

While environmental factors are important in diagnosis, diabetes has been shown to have a genetic component regarding its prevalence in the global population. More specifically, while genetics influences both classifications, type II diabetes mellitus has a stronger genetic association than type I diabetes. Individuals with one type II diabetic parent have a 40% chance of developing the disease while those with two diabetic parents have a 70% chance of

developing diabetes in their lifetime (Ali, O., 2013). In addition, this heritability variates with respect to imprinting as the risk of type II diabetes mellitus is higher if the patient's mother is diabetic when in comparison to those with diabetic fathers (Lyssenko, V., et al., 2015).

Adipokines are a group of signaling proteins acting as the endocrine mediator of adipose tissue cells, the importance of which are becoming readily apparent with increasing research (Kwoon, H., & Pessin, J., 2013). The adipocytokine adiponectin (ACRP30) has emerged as a potential amelioration for type II diabetes as insulin resistance is combated in contrast to absolute insulin deficiency as seen in Type I diabetes. Exogenous adiponectin supplementation in humans and rodents has shown insulin-sensitizing, anti-inflammatory, and anti-atherogenic characteristics (Achari, A. E., & Jain, S. K., 2017). According to Lebovitz, "Insulin resistance is defined clinically as the inability of a known quantity of exogenous or endogenous insulin to increase glucose uptake and utilization in an individual as much as it does in the normal population." The body, through the negative effects of lipid accumulation, inflammation, and metabolic overload, has come to have a decreased response to insulin referred to as insulin resistance (Maahs, D. M., West, N. A., Lawrence, J. M., & Mayer-Davis, E. J. 2010 and Muoio, D., Newgard, C. (2008). To combat diabetes and help control glucose levels, increasing the body's sensitivity to insulin is paramount from a treatment standpoint (Achari, A. E., & Jain, S. K., 2017).

REVIEW OF THE LITERATURE

Definition and Presentation of Type II Diabetes Mellitus

Diabetes Mellitus is a group of metabolic diseases with the presentation of persistent hyperglycemia and metabolic abnormalities secondary to the inadequacy of the availability or body's sensitivity to insulin (Kharroubi, A. T., & Darwish, H. M., 2015). More than 90-95% of diabetics have the Type II classification (Kharroubi, A. T., & Darwish, H. M., 2015). Insulin resistance is held as the primary accountable construct for the onset of Type II diabetes mellitus. This resistance in type II, non-insulin dependent diabetes mellitus (NIDDM), patients increases the total demand for insulin required for physiological glucose uptake. Insulin production increases initially to meet increasing demand, yet, over time, production decreases as pancreatic β cells undergo change trying to keep up with supraphysiological demand (Goyal, R., & Jialal I., 2021). In fact, β cell mass and function in type II diabetics is significantly decreased over time which can eventually lead to patient dependency on exogenous insulin (Sakuraba, H., et al., 2002). Yet, unlike other categories of diabetes, NIDDM patients do not have to be dependent on insulin (Kharroubi, A. T., & Darwish, H. M., 2015).

Type II diabetes is most prevalent in individuals over 45 years old with a recent upward trend in children and young adults. This has been attributed to the increasing prevalence of sedentary lifestyles, obesity, and high fat diets. In fact, most patients with NIDDM are obese or possess a high percentage of body fat (Goyal, R., & Jialal I., 2021). While previously stated, though a large portion of diabetes presentation is due to environmental and lifestyle factors, genetic heritability is important to consider in the process of screening and potential diagnosis of diabetes mellitus (Lyssenko, V., et al., 2015).

The presentation of type II diabetes mellitus includes polydipsia, polyuria, fatigue, and the slow healing of wounds (Goyal, R., & Jialal I., 2021). Initial presentation of slight hyperglycemia can become severe due to infection or stress. This severe hyperglycemia can lead to a presentation of ketoacidosis or nonketotic hyperosmolar syndrome in these diabetic patients (Goyal, R., & Jialal I., 2021).

It is important to recognize in this discussion that while insulin resistance is a defining trait of type II diabetes, not all patients with insulin resistance are type II diabetic. Therefore, a general knowledge of diagnostic criteria is paramount.

Diabetes Diagnosis

Chronic hyperglycemia is key for the diagnosis of NIDDM (Seino, Y., et al., 2010). Diabetes diagnosis requires one of the following in patient presentation: a fasting glucose ≥126 mg/dl, a two-hour post 75 gram oral glucose tolerance test (OGTT) ≥200 mg/dl, or a nonfasting casual glucose of ≥200 mg/dl (Seino, Y., Nanjo, K., Tajima, N. et al., 2010).

Mechanisms of Insulin Resistance

Generally, aberrations in insulin receptor substrate one (IRS-1) and insulin receptor substrate two (IRS-2) pathways have been heavily explored as to their contribution to the development of insulin resistance (Kharroubi, A. T., & Darwish, H. M., 2015). IRS proteins seem to be cornerstone to intracellular insulin response. Decreased levels of these IRS signaling proteins have been implicated in the attribution of insulin resistance in both rodents and humans (Kwon, H., & Pessin, J. E., 2013). Abnormalities in IRS signaling can arise from aberrant phosphorylation of Protein Kinase B (AKT) and Protein Kinase C (PKC) (Muoio, D., Newgard, C., 2008). Notably, AMP-activated protein kinase, c-Jun N-terminal protein kinase, as well as G-

Protein coupled receptor (GPCR) kinase 2 are not dependent on insulin but have been shown to inappropriately phosphorylate substrates in the insulin pathway in an insulin resistant state (Muoio, D., Newgard, C., 2008).

As a whole, elevated free fatty acid levels in plasma are associated with decreased insulin sensitivity (Satoh, H. et al., 2005). Plasma nonesterified fatty acids (FFA) have been identified as the primary vehicle for lipid circulation in plasma (Satoh, H. et al., 2005). The primary source of fatty acid liberation into plasma is directly from adipose tissue. Since it is well accepted that high levels of free fatty acids in the bloodstream lead to adverse metabolic complications, it is feasible that increasing levels of adipocytes and the associated circulation of lipids may lead to the development of insulin resistance (Satoh, H. et al., 2005). In the liver, insulin resistance is thought to be secondary to the gathering of fatty acids due to a deficiency in their oxidation and breakdown (Muoio, D., Newgard, C., 2008). Intracellularly, with excesses of FFAs, normal oxidation cannot be properly performed, resulting in the abnormal conversion of these fats into diacylglycerols, triglycerides, and ceramides, the presence of which positively correlate to insulin resistance through interaction with insulin signaling kinases (Muoio, D., Newgard, C., 2008). In muscle tissue, similar theories are seen wherein levels of fatty acids and their peroxidative by-products are strongly correlated to insulin resistance (Muoio, D., Newgard, C., 2008, and Shulman, G. I., 2000).

Clearly, increasing concentrations of fatty acids both intracellularly and in circulation are directly correlated with insulin resistance. Other theories exist that this intramuscular insulin resistance can result from by-products of inappropriate lipid oxidation and lipid accumulation in the mitochondria (Muoio, D., Newgard, C., 2008). In addition, it has been suggested that

impairment of metabolic flexibility, the capacity to adapt substrate oxidation rates in response to changes in fuel availability, is a key indicator and contributor to insulin resistance (Shulman, G. I., 2000, and Rynders, C. A., Blanc, S., Dejong, N., Bessesen, D. H., & Bergouignan, A., 2017). Clearly, a whole-body approach to metabolism is necessary to fully elucidate its contribution to insulin resistance. It is quite evident that insulin resistance does not arise from a singular cellular mechanical failure, rather, it is the consequence of multiple aberrations in direct insulin dependent and non-dependent pathways.

Adipocytes

Previously thought as only a storage cell, adipose tissue has demonstrated importance in maintaining bodily homeostasis (Rosen, E., & Spiegelman, B., 2006). Adipocytes are clearly involved in the processes of nutrient homeostasis, immune response, blood pressure regulation and inflammation. Specifically, it is now clear that adipocytes have an undeniable effect on glucose homeostasis and must be considered in the treatment of diabetes (Rosen, E., & Spiegelman, B., 2006). More specifically, adipocytes can control and regulate the homeostasis and metabolism of fatty acids and sugar through the secretion of adipokines, a group of bioactive peptides (Guilherme, A., Virbasius, J. V., Puri, V., & Czech, M. P., 2008). Among these are leptin, tumor necrosis factor alpha (TNFα), interleukin 6 (IL-6), and adiponectin. The majority of adipokines are pro-inflammatory and positively correlate with adiposity. Other anti-inflammatory adipokines such as adiponectin have a negative correlation with adiposity (Guilherme, A., Virbasius, J. V., Puri, V., & Czech, M. P., 2008). It has been demonstrated that visceral and subcutaneous adipose tissue vary in their ability to secrete adipokines thereby

regulating inflammation and insulin resistance (Guilherme, A., Virbasius, J. V., Puri, V., & Czech, M. P., 2008).

Adipokines

As the endocrine mediator of adipose tissue, the effects of adipokines (adipocytokines) are incredibly diverse (Kwon, H., & Pessin, J. E., 2013). Chemerin is highly expressed in white adipose tissue and showcases anti-inflammatory effects while also promoting adipocyte differentiation. While not thoroughly as explored as adiponectin in this work, chemerin has also been shown to enhance insulin receptor responsiveness. (Satoh, H., et al., 2005). Yet, chemerin levels have not been shown to be greatly differentiated between human controls and those individuals with type II diabetes mellitus. Leptin is one of the earliest discovered adipocytokines and has shown essential activity in the regulation of food intake via hypothalamic activity (Satoh, H., et al., 2005). Yet, it has become clear that leptin functions in a different manner when it comes to the direct regulation of glycemic levels (Muoio, D., Newgard, C. (2008). Leptin has also shown peripheral insulin sensitizing properties in certain cases. Yet, leptin resistance, in which the increasing levels of leptin witnessed in insulin resistant individuals exhibit a diminished bodily response due to the greater than normal presence of the protein itself, hampers the effectiveness of leptin as a supplementary compound (Muoio, D., Newgard, C., 2008).

The function of the adipokine omentin needs to be further elucidated, but it may enhance the body's response to insulin. A definite inverse correlation has been shown regarding omentin levels in relation to obesity (Muoio, D., & Newgard, C., 2008). Yet, too little is known about the function of omentin to state with any degree of certainty that supplementation of this adipokine could resolve, at least temporarily, a state of insulin resistance.

Resistin, while shrouded in controversy, has been hypothesized to be a link between inflammatory mediators and metabolic modulators (Muoio, D., & Newgard, C., 2008). Showing a direct correlation with adiposity and lipid levels in mouse models, resistin is a clear exclusion from any sort of supplementary therapy to treat diabetes mellitus (Rajala, M. W., et al., 2004).

Elevated levels of retinol binding protein 4 (RBP4) have been presented in humans and mice concurrently to obese and insulin resistant states. In fact, overexpression of RBP4 was accompanied by insulin signaling impairment in mouse model studies (Muoio, D., & Newgard, C., 2008). Thus, supplementation of RBP4 would not enhance insulin signaling. While the direct correlation between retinol binding protein and negative metabolic complications remains clear, further research needs to be performed to better elucidate the mechanisms and specific actions of RBP4 (Muoio, D., & Newgard, C., 2008).

Tumor Necrosis Factor alpha concentrations are directly correlated with obesity. In fact, deletion of TNF-alpha receptors improves insulin sensitivity in obese mice (Adamczak, M. et al., 2004). It has been hypothesized that TNF-alpha overexpression blocks the action of insulin in the body. This claim has been supported via the same mechanism in cultured cells (Adamczak, M. et al., 2004).

Even with all that is known about adipokines, conflicting data and a lack of clarity regarding their mechanisms justifies the need for more research into adipokines and their interplay.

Inflammation

In-vivo studies in mice seem to show a relationship between insulin resistance and chronic inflammation (Arkan, M., Hevener, A., Greten, F. et al., 2005). Mice with a

constitutively functioning Inhibitor of Nuclear Factor Kappa B subunit beta (IKKβ), an activator of the inflammatory mediator NF-kB (Liu, T., et al., 2017), developed glucose intolerance and a state of insulin resistance (Arkan, M., Hevener, A., Greten, F. et al., 2005). In mice with IKKβ knockout in hepatocytes, liver responsiveness to insulin was retained with a high fat diet (HFD), but peripheral resistance to insulin was developed. With the deletion of IKKβ in myeloid cells, systemic insulin sensitivity improved. As such, myeloid cells were suggested to be the mediator of communication between insulin responsive tissues that utilize proinflammatory cytokines (Arkan, M., Hevener, A., Greten, F. et al., 2005). Either way, it is clear from animal models that inflammation pathways underlie and greatly affect insulin responsiveness.

Adiponectin

The most abundant protein secreted by adipose tissue, the signaling protein adiponectin (ACRP30) is secreted primarily by white adipose cells (Rabe, K., Lehrke, M., Parhofer, K. G., & Broedl, U. C. 2008, and Achari, A. E., & Jain, S. K., 2017). ACRP30 is also expressed by osteoblasts, liver parenchyma cells, myocytes, and epithelial cells in comparatively low levels (Achari, A. E., & Jain, S. K., 2017). The loci 3q27 is the responsible gene coding region for ACRP30 (Khoramipour, K., 2021). From adipocytes, adiponectin can be secreted in three different forms, a 67 kDa trimer, a 140 kDa hexamer, and a 300 kDa multimer also known as high molecular weight adiponectin (HMW) (Achari, A. E., & Jain, S. K., 2017). This HMW complex of adiponectin consists of a globular region, collagenous domain, species-specific region, and signal peptide. The full-length form of adiponectin, along with its globular form derived from proteolysis of the multimer, are the primarily vehicles for the protective effects of adiponectin. (Palanivel, R., et al., 2007).

Adiponectin has two major receptors, Adiponectin Receptor 1 (AdipoR1) and AdipoR2 (Yamauchi, T., et al., 2007). Both receptors are of similar structure with seven transmembrane domains (Achari, A. E., & Jain, S. K., 2017). In addition, T-Cadherin has adiponectin binding properties specifically for HMW adiponectin (Achari, A. E., & Jain, S. K., 2017). AdipoR1 is expressed most prominently in skeletal myocytes and has a greater affinity for globular adiponectin than its HMW multimer (Yamauchi, T., et al., 2007). In contrast, AdipoR2 is expressed most concentrated in the liver with the preferred ligand of HMW ACRP30 (Yamauchi, T., et al., 2007). As previously mentioned, adiponectin has been reported to have significant insulin-sensitizing and anti-atherogenic properties in vivo mice (Okamoto, Y., et al, 2002). Thus, research has been conducted to elucidate the intracellular signaling of adiponectin via its receptors, AdipoR1 and AdipoR2. Adiponectin has been reported to activate AMP activated protein kinase through the direct mediation of APPL1 (Achari, A. E., & Jain, S. K., 2017). AMP activated kinase has been shown to contribute to the cell's energy metabolism, including the oxidation of hepatic cell fats in vivo through the action of Acetyl-CoA carboxylase (ACC) (Xu, A., et al., 2003 and Foretz, et al., 2018). Adiponectin has also been shown to increase the expression of peroxisome proliferator-activated receptor alpha (PPAR-α), a transcriptional factor heavily involved with energy homeostasis and fatty acid oxidation (Achari, A. E., & Jain, S. K., 2017). Adiponectin levels are inversely correlated with obesity and insulin resistance in animal models (Rabe, K., Lehrke, M., Parhofer, K. G., & Broedl, U. C. (2008). Additionally, adiponectin infusion increased glucose uptake, myocytic fat oxidation and decreased glucose production in mice (Rabe, K., Lehrke, M., Parhofer, K. G., & Broedl, U. C. (2008). Considering the implications of fatty acid accumulation in insulin resistance, it is plausible that ACRP30 has

anti-diabetic properties.

Recently, it has become more evident that single nucleotide polymorphisms, specifically a thymine in place of a guanine at the +276 locus in the adiponectin locus, may be associated with increased body mass index (BMI) and type II diabetes, (Rabe, K., Lehrke, M., Parhofer, K. G., & Broedl, U. C. (2008).

Adiponectin Action in Hepatocytes

It has been implied through in-vivo observations that at least part of the glucose lowering abilities of adiponectin comes from its ability to decrease endogenous glucose production (EGP) in the liver (Stefan, N., et al., 2003). This lowering of EGP was seen with a decrease in phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) activity, both heavily involved in hepatic gluconeogenic activity. (Stefan, N., et al., 2003, and Zhang, X., et al., 2019). In addition, adiponectin has been pointed to as a potential amelioration of steatosis in the liver. This action of reducing fatty content in hepatocytes is a result of modified acetyl-coA-carboxylase (ACC) and fatty acid synthase activity (FAS). Both ACC and FAS are important in fatty acid synthesis and oxidation (Xu, A., et al, 2003). In addition, ACRP30 supplementation has shown an increase in hepatic fatty acid oxidation through the modulation of carnitine palmitoyltransferase 1 activity (Xu, A., et al, 2003).

Adiponectin Action in Skeletal Muscle

It has been demonstrated that adiponectin has a significant effect on glucose uptake and fatty acid oxidation in skeletal muscle cells. Globular adiponectin has been shown to increase glucose uptake in rat myocytes (Ceddia, R. B., et al., 2005). This increase in uptake is a consequence of increased GLUT4 translocation (Ceddia, R. B., et al., 2005). Interestingly, in the

same study, adiponectin decreased glycogen synthesis in muscle cells along with the increased production of lactate (Ceddia, R. B., et al., 2005). Increased GLUT4 translocation has been shown to be a product of ACRP30 induced activation of MAPK and AMPK pathways (Achari, A. E., & Jain, S. K., 2017). Fatty acid oxidation, much like in the liver, is witnessed in skeletal muscle cells because of AMPK activation (Achari, A. E., & Jain, S. K., 2017). In summation, adiponectin has been shown to increase GLUT4 translocation, increase glucose uptake, and increase fatty acid oxidation in the muscle cells of model organisms (Achari, A. E., & Jain, S. K., 2017).

Physiological and pathophysiological concentrations of Adiponectin

Adiponectin levels have been shown to be significantly decreased in populations of diabetic individuals in comparison to their non-diabetic counterparts. Levels of diabetic men (6.6+/-0.4 μg/ml) were lower than non-diabetic males (7.9+/-0.5 μg/mL). A similar correlation was seen in diabetic versus control women, 7.6+/-0.7 μg/mL and 11/7+/-1.0 μg/mL respectively. This deficiency seen in the diabetic patients supports the notion that adiponectin supplementation in diabetic patients may increase health outcomes (Hotta, K., et al., 2000). In addition, this deficiency has been found to contribute to cardiovascular complications such as macroangiopathy (Hotta, K., et al., 2000).

Existing Therapeutics for Type II Diabetes Mellitus

Insulin supplementation has prevailed as one of the most powerful treatment methods for controlling hyperglycemia (Kharroubi, A. T., & Darwish, H. M. 2015). Insulin therapy has been shown to be the most potent and cost-effective methodology regarding the treatment of diabetes mellitus. Treatment with exogenous insulin early in the treatment of Type II Diabetes has been

shown to lower glycemic levels and macrovascular risk (Kharroubi, A. T., & Darwish, H. M. 2015). While this method may be viewed with hesitancy, Weng et al. showcased the ability of early, intensive insulin therapy to maintain and restore β-cell function (Weng, J., et al., 2008). The incidence of hypoglycemic episodes of varying intensity are increased with the utilization of insulin supplementation (Tomky, D., 2005). These hypoglycemic episodes, specifically in patients with insulin dependent diabetes mellitus (IDDM) and NIDDM, have been linked to short term deleterious effects and higher incidence of cardiovascular events (Kreider, K.E., et al., 2017).

Metformin, a biguanide analog, is the most prescribed drug for the treatment of type II diabetes (Horakova, O., et al., 2019). Metformin has been shown to reduce glucose levels in the bloodstream via the suppression of glucose production in hepatocytes (Horakova, O., et al., 2019). It has also been proposed that Metformin reduces hyperglycemia by inducing greater glucose uptake in skeletal myocytes, yet this is not fully understood (Horakova, O., et al., 2019). Metformin has proven to be an effective option for long-term glycemic control (Horakova, O., et al., 2019).

Metformin was theorized to utilize adiponectin to exert its metabolic effects, yet recent research has concluded that adiponectin is not involved in metformin's insulin-sensitizing activities (Fujita. H., et al., 2005). Metformin has shown the ability to reverse pancreatic beta cell dysfunction due to chronic FFA exposure (Patanè, G., et al., 2000). Like adiponectin, metformin has been shown to lower FFA levels (Patanè, G., et al., 2000). It is well understood that metformin monotherapy is a relatively well tolerated approach resulting in good glycemic control for human NIDDM patients (Defronzo, R. A., et al., 1995).

Thiazolidinediones

Thiazolidinediones (TZDs), a drug class that acts as a PPAR gamma agonist, have been utilized as a pharmacological treatment for diabetes (Achari, A. E., & Jain S. K., 2017). TZDs have been shown to increase circulating adiponectin concentration (Achari, A. E., & Jain S. K., 2017). As expected, TZDs, such as rosiglitazone, have been shown to increase insulin-sensitivity via an uptick in serum adiponectin levels in obese mice alongside no improvement in insulin sensitization in adiponectin-null mice (Kubota, N., et al., 2006). The proposed mechanism for this increasing insulin sensitivity is through the acceleration of adiponectin production (Achari, A. E., & Jain S. K., 2017). More specifically, TZDs lower blood sugar by increasing glucose uptake and insulin sensitivity in skeletal muscle. This increase in adiponectin concentration, as supported by prior studies, has also increased glucose tolerance and ameliorated hyperglycemia (Achari, A. E., & Jain S. K., 2017). Unfortunately, thiazolidinediones have been linked to increased fractures, cardiovascular events, edema, and weight gain from notable studies, including one from the US Food and Drug Administration (FDA) (Lipscombe L. L., 2009). As a result, the FDA has added a black box warning to TZD class drugs, including pioglitazone and rosiglitazone (Achari, A. E., & Jain S. K., 2017).

Combination Therapeutics

While certain assays have proved effective alone, combination therapeutics may pose an effective solution to better glycemic control. In a recent study conducted on Type II diabetic youth, a combination of rosiglitazone and metformin lead to better long-term control of glycemic levels in comparison to metformin alone (Today, 2007). In addition, treatment failure of the combination group was significantly lower than either drug alone (Today, 2007).

Geriatric Considerations

Almost half of individuals with type II diabetes are above the age of 60 (Adamczak, M. et al., 2004). Therefore, therapeutic compounds should be well tolerated in the geriatric population. With the possibility of complications higher in this population, hypoglycemic episodes should be avoided in tandem with the consideration of liver and kidney function (Yakaryilmaz, F. D., and Öztürk, Z. A. 2017). In fact, metformin usage is not recommended in geriatric cases with limited kidney function (Adamczak, M. et al., 2004). In these cases, insulin therapy is often utilized with frequent monitoring for hypoglycemia (Adamczak, M. et al., 2004). Yet, geriatric patients with a glomerular filtration rate greater than sixty milliliters a minute are indicated for metformin use. In fact, even with certain contraindications, metformin remains ever popular in the treatment of geriatric type II diabetes mellitus (Yakaryilmaz, F. D., and Öztürk, Z. A. 2017).

Summary

Diabetes Mellitus is a public health crisis that shows no intention of slowing down (Kharroubi, A. T., & Darwish, H. M., 2015). While multiple therapeutics exists, side effects are prominent, and failure of glycemic control can still occur. Adiponectin, an adipocytokine, (ACRP30) shows anti-atherogenic and insulin sensitizing properties in mice models. In addition, adiponectin levels have been described as deficient across diabetic and obese patients, suggesting potential benefits with the use of therapeutic supplementation. In the following meta-analysis, the feasibility and efficacy of ACRP30 as a supplementary therapeutic for the treatment of Type II diabetes mellitus will be explored.

METHODS

A search for like-minded studies written between 2000 and 2021 was conducted using PubMed and Google Scholar. The search terms for the effects of exogenous ACRP30 on insulin resistance are as follows: “exogenous adiponectin” OR “exogenous adiponectin mice” OR “adiponectin glucose tolerance” OR “adiponectin insulin resistance” OR “adiponectin supplementation”. The search terms for the effects of Metformin on insulin resistance are as follows: “Metformin glucose tolerance” OR “Metformin insulin resistance” OR “Metformin mice insulin resistance”.

Out of all potential studies, inclusion required the fulfillment of the following requirements showcased in Table 1.

Inclusion Criteria:
• Mouse Model • Mice previously fed HFD or obese mice. • Quantitative measurement of glucose tolerance test (table or graph) • Quantitative measurement of fasting glucose of HFD or obese mice. • Presence of Metformin usage or adiponectin supplementation in mice before fasting glucose or GTT measurements. • Quantitative presentation of HFD or obese control.

Table 1. Inclusion Criteria

Data Analysis

To elucidate the effects of adiponectin supplementation and metformin on insulin resistant states in HFD or obese mice, area under the curve (AUC) measurements will be utilized on GTT data to compare glycemic levels between the therapeutic and control group of each study, then compared in a larger analysis to each other. This AUC measurement will elucidate glycemic control in response to a glucose load such as at mealtime or in the presence of stressors.

Fasting glucose and fasting insulin measurements will be compared across control and therapeutic groups for both metformin and adiponectin.

The traditional trapezoidal method will be utilized to derive AUC values.

When available, direct data from tables will be utilized to conduct analyses. If not available, WebPlotDigitzer will be utilized to extract data from available graphs. While, not as accurate as directly presented numbers, data will be accurate enough to make general observations on efficacy.

RESULTS

Included Studies

As seen in Figure 1, included studies had to meet inclusion criteria while maintaining similar environments between therapeutic and control groups. Dosing could not be kept constant. This will be explored more in the discussion section. In addition, GTT values are all reasonable but not controlled, see Table 2 below. The period in which fasting bloodwork was obtained was somewhat variable. GTT findings were all performed after weeks of treatment.

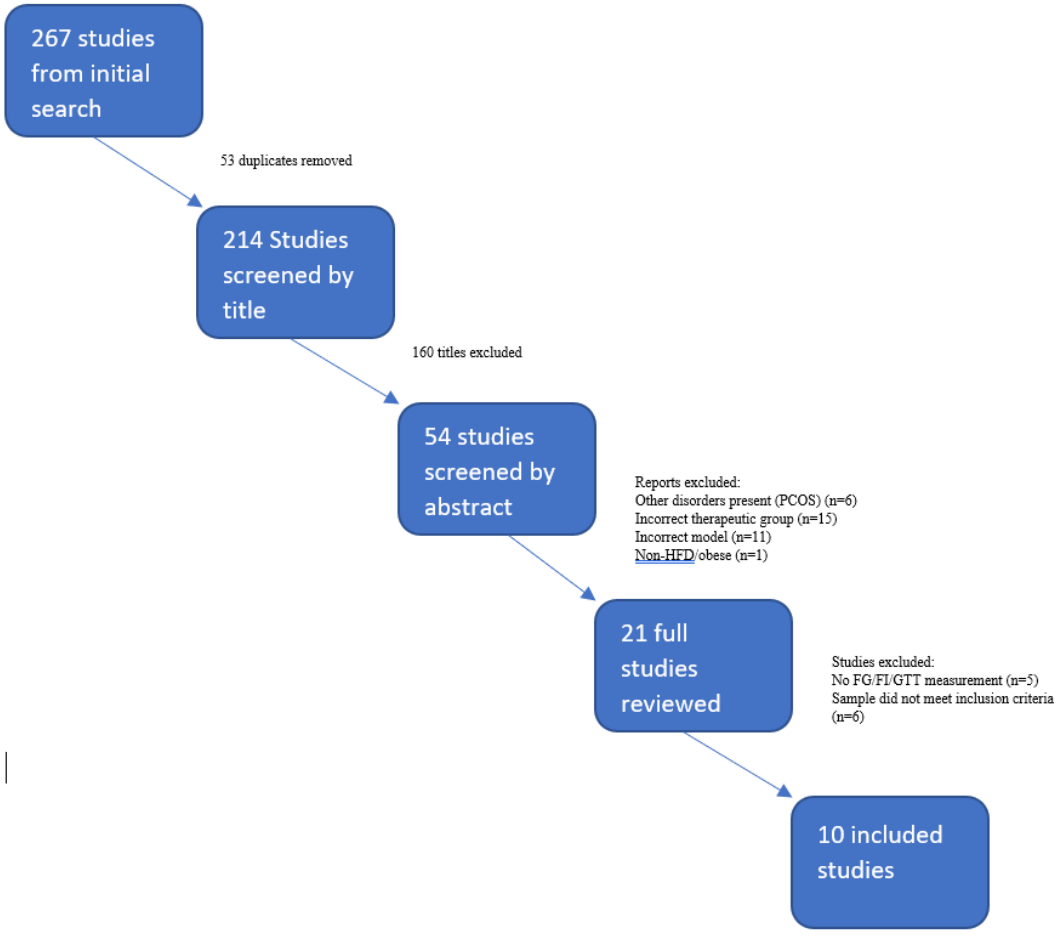

Figure 1. Inclusion Review Process

Author	Therapeutic Group	Values Tested	Findings
Li et al., 2020	Adiponectin (n=10)	Fasting Glucose (FG), Fasting Insulin (FI)	Therapeutic FG, FI reduced from control
Otabe et al., 2007	Adiponectin line 11 (n=6)	Fasting Glucose (FG), Fasting Insulin (FI)	Therapeutic FG, FI reduced from control
Otabe et al., 2007	Adiponectin line 13 (n=6)	Fasting Glucose (FG), Fasting Insulin (FI)	Therapeutic FG, FI reduced from control
Kandasamy et al., 2012	Adiponectin (n=6)	Fasting Glucose (FG), Fasting Insulin (FI), GTT (2g/kg)	Therapeutic FG, FI, AUC reduced from control
Koch et al., 2014	Adiponectin (n=9-11) Control (n=9-11)	GTT (1g/kg)	AUC reduced from control
Kim et al., 2007	Adiponectin (n=5) Control (n=5)	Fasting Glucose (FG), Fasting Insulin (FI), GTT (2.5g/kg)	Therapeutic FG, FI, AUC reduced from control
Matsui et al., 2010	Metformin .25% (n=7) Control (n=7)	Fasting Glucose (FG), Fasting Insulin (FI), GTT (2g/kg)	Therapeutic FG, FI, AUC reduced from control
Matsui et al., 2010	Metformin .50%(n=7) Control (n=7)	Fasting Glucose (FG), Fasting Insulin (FI), GTT (2g/kg)	Therapeutic FG, FI, AUC reduced from control
Horakova et al., 2019	Metformin 60 mg/kg (n=4-5)	Fasting Glucose (FG), GTT (3g/kg)	Therapeutic FG, AUC reduced from control,
Horakova et al., 2019	Metformin 200 mg/kg (n=4-5)	Fasting Glucose (FG), GTT (3g/kg)	Therapeutic FG, AUC reduced from control
Horakova et al., 2019	Metformin 400 mg/kg (n=4-5)	Fasting Glucose (FG), GTT (3g/kg)	Therapeutic FG, AUC reduced from control
Derkach et al., 2019	Metformin 200 mg/kg (n=15)	Fasting Glucose (FG), Fasting Insulin (FI), GTT (2g/kg)	Therapeutic FG, FI, AUC reduced from control
Derkach et al., 2019	Metformin 400 mg/kg (n=15)	Fasting Glucose (FG), Fasting Insulin (FI), GTT (2g/kg)	Therapeutic FG, FI, AUC reduced from control
Derkach et al., 2019	Metformin 600 mg/kg (n=10)	Fasting Glucose (FG), Fasting Insulin (FI), GTT (2g/kg)	Therapeutic FG, FI, AUC reduced from control
Zhou et al., 2016	Metformin (n=4-6)	Fasting Glucose (FG), Fasting Insulin (FI), GTT (1.5g/kg)	Therapeutic FG, FI, AUC reduced from control
Zabielski et al., 2018	Metformin (n=8)	Fasting Glucose (FG), GTT (3g/kg)	Therapeutic FG, AUC reduced from control

Table 2. Included Studies

Adiponectin and Metformin effects on fasting glucose and insulin

Among analyzed groups of mice models, a 22% reduction of fasting blood glucose was observed in mice experiencing long-term hyperadiponectinemia in comparison to high fat diet controls. Fasting insulin in the same group comparison observed a 64% reduction. While fasting glucose was consistently higher than normal diet mice without obesity, the reduction due to adiponectin administration was significant. In analyzed groups of mice models administered with various metformin dosages, a 14% reduction of fasting blood glucose was observed in comparison to a high fat diet control. Similarly to the adiponectin group, a 30% reduction in fasting insulin was witnessed.

Effects of Adiponectin and Metformin Administration on AUC for Glucose Tolerance Tests

Among collected glucose tolerance tests, a 41% reduction was witnessed in compiled therapeutic groups from adiponectin. A 34% reduction was witnessed in groups with Metformin administration.

For specific reductions in fasting glucose values, see Table 3. For specific reductions in fasting insulin values, see Table 4. For specific reductions in GTT AUC values, see Table 5.

Compound/Dosage	Fasting Glucose Percent Reduction
Adiponectin 2.5 mcg/day	15.87%
Adiponectin, transgene expression	30.15%
Adiponectin, transgene expression	20.59%
Adiponectin, transgene expression	-5.10%
Adiponectin, mouse plasmid DNA	15.63
Adiponectin, 1 mcg/day	9.22%
Adiponectin, transgenic expression	67.94%
Metformin .25% in HFD	57.27%
Metformin .50% in HFD	59.09%
Metformin 200 mg/kg	3%
Metformin 400 mg/kg	13%

Metformin 600 mg/kg	19%
Metformin 100 mg/kg	14%
Metformin 300 mg/kg	20.57%

Table 3. Effects of adiponectin and metformin on reduction in fasting glucose values

Compound/Dosage	Fasting Insulin Percent Reduction
Adiponectin 2.5 mcg/day	70.71%
Adiponectin, transgene expression	88.77%
Adiponectin, transgene expression	39.30%
Adiponectin, mouse plasmid DNA	58.54%
Adiponectin, transgenic expression	70.32%
Metformin .25% in HFD	32.20%
Metformin .50% in HFD	21.54%
Metformin 200 mg/kg	22.02%
Metformin 400 mg/kg	19.27%
Metformin 600 mg/kg	42.20%
Metformin 100 mg/kg	43.35%

Table 4. Effects of adiponectin and metformin on reduction in fasting insulin values

Adiponectin, mouse plasmid DNA	49.79%
Adiponectin, 1 mcg/day	24.65%
Adiponectin, transgenic expression	56.76%
Adiponectin, 1 mcg	32.99%
Metformin, .25% in HFD	40.15%
Metformin, .5%	47.83%
Metformin, 60 mg/kg	16.63%
Metformin, 200 mg/kg	30.22%
Metformin, 400 mg/kg	45.23%
Metformin, 200 mg/kg	30.81%
Metformin, 400 mg/kg	31.05%
Metformin, 600 mg/kg	48.55%
Metformin, 100 mg/kg	33.69%
Metformin, 300 mg/kg	20.57%

Table 5. Effect of adiponectin and metformin on reduction in GTT AUC values

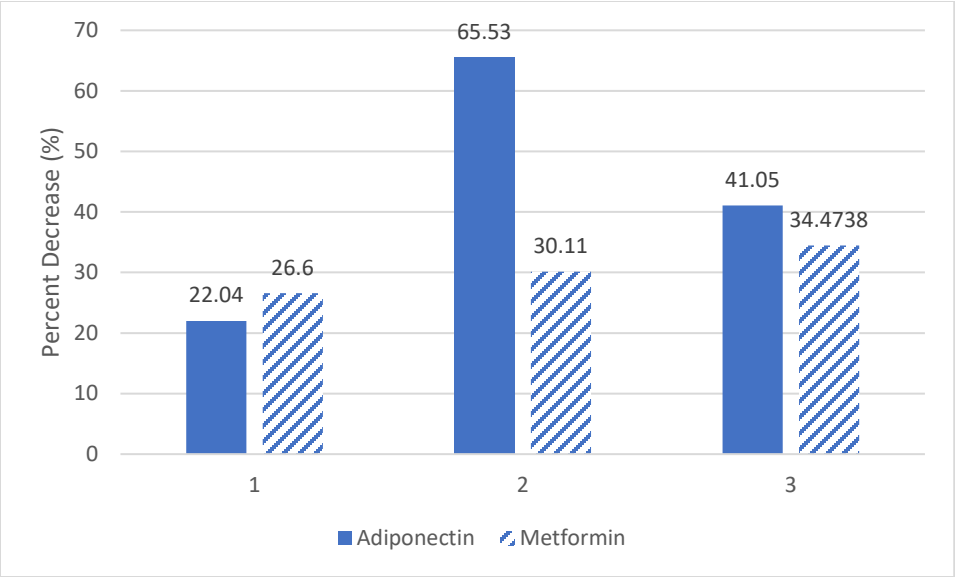

Figure 2. Percent decrease of adiponectin (solid) and metformin (hatched), 1) fasting glucose 2) fasting insulin 3) glucose AUC

DISCUSSION

Contextualizing the data, it is quite clear that adiponectin has significant glucose homeostatic properties. Upon supplementation with ACRP30, whether upregulated genetically or through injection, mice with supraphysiological concentrations of adiponectin had consistently lower fasting glucose and fasting insulin values than their non-supplemented high fat diet control counterparts.

With a 22.04 % reduction in fasting glucose among high fat diet mice when compared to non-therapeutic controls, adiponectin has significant efficacy keeping glycemic concentrations from reaching pathophysiological levels in insulin resistant and diabetic states. This efficacy becomes quite clear when compared to the 26.6% reduction in fasting glucose in mice treated with metformin.

Elucidating effect on fasting glucose alone is not enough to justify the therapeutic potential of adiponectin. Utilizing the same organisms seen in the preceding values, a 65.53% reduction in fasting insulin values was present in mice with adiponectin supplementation in comparison to high fat diet controls. Considering these values were gathered simultaneously across all analyzed data, a reduction in fasting glucose values in tandem with greatly reduced plasma insulin values suggests that the body's response to insulin has been greatly sensitized. With a 22.04% reduction in fasting glucose and 65.53% reduction in fasting insulin, the insulin sensitizing properties of the adipokine adiponectin seem to be significant in the basal state.

Yet, a physiological basal state is not constant for the human individual. Homeostatic properties change in response to intake. Therefore, glucose tolerance tests were utilized to illuminate adiponectin's effectiveness at controlling blood sugar spikes in response to a bolus of

glucose, such as in a high carbohydrate meal. Compared to a high fat diet control, mice with a state of hyperadiponectinemia had an average area under the curve reduction of 41.05% in a two-hour GTT. Therefore, supplementing with adiponectin to reach normal to excess levels to combat ACRP30 deficiency was effective and significant at controlling near normal post-bolus blood sugar levels.

In isolation, it is difficult to make clear the significance of these values. Metformin was utilized as a standard of care (SOC) to help illustrate the potential anti-diabetic activities of adiponectin against the most prescribed type II anti-diabetic drug.

On high fat diet mice under similar physiological conditions to that of the mice from the adiponectin trials, a 26.6% reduction in blood glucose was witnessed as mentioned previously in tandem with a 30.11% reduction in blood insulin values. It is quite clear that metformin has significant anti-diabetic properties, effectively lowering basal blood sugar while also lowering blood insulin values. To illustrate therapeutic metformin's response to a sugar bolus, a two-hour glucose tolerance test was utilized. Compared to control group high fat diet mice, metformin treated mice had a 34.47% reduction in area under the curve value.

Comparatively, as seen in Figure 2, the mice in adiponectin trials experienced a lesser reduction of fasting blood glucose and greater reduction of fasting blood insulin values than metformin mice. Furthermore, adiponectin supplemented mice had a greater reduction in total AUC values over control groups than the metformin trial mice.

This data in isolation supports the notion that insulin resistant mice experienced an alleviation of this relative deficiency of insulin in response to an uptick of serum adiponectin concentration whether it be from injection or overexpression of adiponectin. The extent of this

amelioration performed similarly to the leading standard of care in this specific analysis.

It is quite clear that adiponectin is an effective anti-diabetic compound and should be considered for further research regarding the adipokines therapeutic potential.

With the current mechanism of insulin resistance being mediated by fatty acid accumulation in cells, it is feasible that adiponectin, a compound heavily involved in proper fatty acid oxidation and glucose uptake, works in support of this theory.

Yet, further research is key. The potential of adiponectin's efficacy is clear with varying dosages of metformin as a benchmark. With the current proposed mechanism that adiponectin both lowers EGP and increases peripheral tissues' insulin sensitivity through the activation of PPAR gamma pathways and ACC pathways, it is feasible that adiponectin supplementation could competently control hyperglycemia.

While this data may make adiponectin seem like a stronger therapeutic candidate than metformin, this data needs to be viewed with hesitancy. Considering the geriatric demand of type II diabetes mellitus treatment, more research certainly needs to be done in geriatric models to elucidate the relative efficacy of adiponectin in this demographic. Adiponectin supplementation may not be a feasible therapeutic in old-age individuals due to the natural increase of adiponectin with age. Comparatively, metformin is generally well tolerated, except in those with impaired renal function (Yakaryilmaz, F. D., & Öztürk, Z. A., 2017).

Aside from geriatric treatment, dosing needs to be considered. Injection and even serum concentrations of adiponectin can be easily measured. Yet, comparing dosing from adiponectin to the standard of care metformin is quite difficult across research with many different dosage measurements. Thus, conclusions to adiponectin's effectiveness cannot be made until studies are

completed with more standardized doses of adiponectin and metformin as well as more standardized and controlled conditions. The prime example of the lack of control in this analysis being the amount of treatment days allowed before test values were collected, as well as glucose bolus quantities.

In fact, while this analysis strongly suggests that adiponectin has the potential to be a therapeutic assay for the treatment of type II diabetes, it is infeasible to definitively say that it is more effective than the SOC due to these dosing limitations. In addition, it is quite untested as to what a long-term state of hyperadiponectinemia will do to the human organism as effects on adipokine interplay are essential to consider. In addition, sex differences in human adiponectin levels could change the perception of adiponectin's effectiveness as physiological adiponectin concentrations in females are varied from that of males.

In order to justify supplementary adiponectin as a potential therapeutic candidate to ameliorate insulin resistance in the treatment of type II diabetes, far more standardized research among model organisms need to be completed before human models can be considered. While the potential is elucidated, dosing effectiveness and long-term viability, as well as geriatric considerations, need to be considered far more seriously before any human supplementation or therapy may begin.

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