FROM:
Matthew Schnur, UCCS Student Cell & Molecular Biology

_____

Dr Robert Melamede, UCCS Chair (retired)

_____

Warren Edson, Esquire

_____

BY HAND

Date Delivered:

Dennis E. Ellis, Executive Director
Colorado Department of Health and Environment
4300 Cherry Creek Drive South
Denver, Colorado 80246-1530

Re: Petition to Add Types 1 and 2 Diabetes Mellitus to List of Debilitating Medical Conditions Pursuant to Colorado Constitution, Article XVIII § 14 and 6 CCR 1006-2

On behalf of the undersigned physicians and patients, we hereby submit the enclosed petition, pursuant to 6 CCR 1006-2, to add both Type 1 and 2 diabetes to the list of debilitating medical conditions for which the medical use of marijuana is authorized under the Colorado Constitution, Article XVIII § 14. 6 CCR 1006-2, Regulation 6, section D states:

"Beginning June 1, 2001, the department shall accept physician or patient petitions to add debilitating medical conditions to the list provided in paragraphs A and B of this regulation. The department shall determine if a public rulemaking hearing to modify this regulation is appropriate, and if so, shall petition the Board of Health to set a date for such hearing within one hundred twenty days of receipt of the patient or physician petition. If the department determines that a public rulemaking hearing is not appropriate, it shall notify the petitioner of its action within one hundred eighty days of receipt of submission of the petition. ***In making its determination, the department will consider whether there is information that the proposed condition is chronic, debilitating, and may be specifically diagnosed, and whether there is scientific evidence that treatment with marijuana may have a beneficial effect."***

I. Introduction:

In the following discussion we intend to prove that diabetes mellitus is a clearly diagnosable disease with specific, easily utilized tests that demonstrate exact parameters for categorization into one of two subtypes. These diagnostic criteria have been developed by the world's leading experts in diabetes research; the World Health Organization (WHO) and American Diabetes Association (ADA). Second, we shall identify symptoms and complications resulting from the chronic progression of this disease. In this section we will also address the evidence demonstrating Types 1 and 2 diabetes mellitus as chronic. Three of the symptoms known to occur in the diabetic state, have already been accepted in 6 CCR 1006-2, as acceptable criteria to recommend the use of medical marijuana for (cachexia, severe nausea, severe pain). On these grounds alone, pursuant to the definition of debilitating medical condition in Regulation 5, section B, the use of medical marijuana for diabetes should be recognized. In addition to alleviation of debilitating symptoms of diabetes mellitus outlined in 6 CCR 1006, the medical use of marijuana can prevent nerve damage, blindness, amputation, ketoacidosis, and insulin resistance; all of which are conditions that further reinforce the concept that diabetes is debilitating. The rest of our discussion shall focus on the scientific research identifying specific molecular mechanisms of therapeutic benefit from intervention of diabetes mellitus with medical marijuana.

As marijuana is a whole plant medicine, each dosage will vary with active ingredients, and thus, will never be allowed in clinical FDA trial. In light of this fact, it would be unreasonable to insist upon clinical trial data for support of this petition. Indeed, if clinical trial data was available, petitions such as these would not need to be written. With this in mind, we intend to demonstrate that medical marijuana "**MAY HAVE A BENEFICIAL EFFECT"** for diabetes by utilizing rodent, human, in vivo, and in vitro studies. While none of these models taken individually can account for substantial therapeutic validity, the culmination of consistent findings between species, data analysis, in vitro cultures, and whole organism studies, characterizes a complex system of hormonal interaction by which cannabinoids benefit diabetes patients at a multitude of levels. Additional benefits in prevention and treatment of microangiopathies, sexual dysfunction, hypertension, inflammation, poor wound healing, ketoacidosis, advanced glycation end products, and gastroparesis will also be addressed. Taken as a whole, this presentation overwhelmingly demonstrates beyond reasonable doubt that Diabetes mellitus meets all necessary criteria for its inclusion into diseases for which medical marijuana be allowed state approval.

II. Diagnosis of Diabetes Mellitus:

According to the WHO, diabetes mellitus is "a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both"[1]. Initial presentation is associated with polyuria, thirst, blurred vision, and weight loss. Criteria for diagnosis have not changed since 1985[2], and involve glucose concentration cut-off values for various biological matrices according to the following guidelines when testing 2h postprandial to a standardized glucose load:

Whole Blood: venous >180mg/dL capillary > 200mg/dL

Type 1 and Type 2 diabetes mellitus are further subsets of classification. Type 1 diabetes mellitus now encompasses the terms juvenile onset and insulin dependent (IDDM), whereas Type 2 now refers to NIDDM and adult onset. Type 1 diabetes mellitus results from complete β-cell destruction and is considered an autoimmune process. Autoantibodies against glutamic acid decarboxylase, a β-cell specific enzyme, are found to exist in up to 95% of Type 1 patients. Type 1 diabetics also have no detectable insulin or C-peptide levels, whereas Type 2 diabetics will still have detectable insulin plasma levels[3]. Type 2 diabetes mellitus is characterized by impaired insulin secretion or impaired insulin signaling[4] [5]. A strong correlation exists between obesity and Type 2 diabetes[6]. More specifically, Type 2 diabetes is correlated to fat tissue density, as Type 2 patients not diagnosed as obese will predominantly still feature excessive fat distribution in the abdominal cavity[7]. Both forms of diabetes mellitus are associated with genetic predispositions[8] [9], and thus when clinical presentation occurs, can be considered a chronic condition. Despite having different etiologies, the WHO recognizes homologous clinical stages of progression of both types of diabetes mellitus. Furthermore, as the next sections statistics demonstrate, the ADA validates extrapolation of epidemiological data from Type1 complications to those of Type 2.

III. Diabetes is Debilitating and Chronic:

The following statistics were taken from the American Diabetes Association (http://www.diabetes.org/diabetes-statistics/complications.jsp), unless otherwise cited, and prove unequivocally that diabetes is a debilitating and chronic condition.

1. Heart disease and stroke

- Heart disease and stroke account for about 65% of deaths in people with diabetes.
- Adults with diabetes have heart disease death rates about 2 to 4 times higher than adults without diabetes.

- The risk for stroke is 2 to 4 times higher and the risk of death from stroke is 2.8 times higher among people with diabetes.
- About 73% of adults with diabetes have blood pressure greater than or equal to 130/80 millimeters of mercury (mm Hg) or use prescription medications for hypertension.

2. Blindness

- Diabetic retinopathy causes 12,000 to 24,000 new cases of blindness each year making diabetes the leading cause of new cases of blindness in adults 20-74 years of age.
- ¾ of all diabetic patients 15+ years have retinopathy[10].
- Nearly all individuals diagnosed before the age of 30 will develop retinopathy within the first 20 years of having the disease[11].

3. **Kidney disease**

- Diabetes is the leading cause of kidney failure, accounting for 44% of new cases in 2002.
- In 2002, 44,400 people with diabetes began treatment for end-stage renal disease (ESRD).
- In 2002, a total of 153,730 people with ESRD due to diabetes were living on chronic dialysis or with a kidney transplant.
- In people with type 1 diabetes, therapy that keeps blood glucose levels as close to normal as possible reduces damage to the kidneys by 35% to 56% (New England Journal of Medicine, September 30, 1993). Experts believe that these results can also be applied to those with type 2 diabetes.

4. **Nervous system disease**

- About 60% to 70% of people with diabetes have mild to severe forms of nervous system damage. The results of such damage include impaired sensation or pain in the feet or hands, slowed digestion of food in the stomach, carpal tunnel syndrome, and other nerve problems.
- Almost 30% of people with diabetes aged 40 years or older have impaired sensation in the feet (i.e., at least one area that lacks feeling).
- Severe forms of diabetic nerve disease are a major contributing cause of lower-extremity amputations.
- More than 60% of nontraumatic lower-limb amputations occur in people with diabetes.
- In 2002, about 82,000 nontraumatic lower-limb amputations were performed in people with diabetes.
- The rate of amputation for people with diabetes is 10 times higher than for people without diabetes.

- Diabetic polyneuropathy was found to occur at a rate of 30% within the diabetic population[12]

5. **Sexual Dysfunction**

- Men with diabetes are 2 times as likely to experience erectile dysfunction as men without diabetes.
- Women with type 1 diabetes are twice as likely to experience prevalence of sexual dysfunction compared with women without diabetes.

6. Other complications

- Uncontrolled diabetes often leads to biochemical imbalances that can cause acute life-threatening events, such as diabetic ketoacidosis and hyperosmolar (nonketotic) coma.
- People with diabetes are more susceptible to many other illnesses and, once they acquire these illnesses, often have worse prognoses. For example, they are more likely to die with pneumonia or influenza than people who do not have diabetes.
- 76% patients surveyed have severe chronic abdominal discomfort, with 29% reporting nausea & vomiting, and 34% having abdominal pain[13].
- Gastroparesis occurs in roughly 50% of all diabetics[14]

7. Evidence for Cachexia in Diabetes Patients

The following excerpt was taken from a clinical study[15] on the only known case of reversibility in nerve conduction damage in diabetes, but also illustrates the severity of the diabetic condition.

"A 36-year-old woman presented with subacute hyperglycemic symptoms. Soon after initiation of insulin therapy and the decline of HbA1c from 14.9 to 5.5%, she developed severe lancinating pain and profound weight loss associated with anorexia, amenorrhea, insomnia, and dehydration. On examination, allodynia was so pronounced that a light touch to her shoulder would cause her to weep. Profound loss of subcutaneous adipose tissue and loss of muscle bulk was evident, such that her weight had decreased from a baseline of 58.3 to 41.8 kg (corresponding to a decrease in BMI from 21 to 15.7 kg/m2). Pain, temperature, and light touch sensation were abnormal in the hands and feet."

8. Preventing diabetes complications

- Studies in the United States and abroad have found that improved glycemic control benefits people with either type 1 or type 2 diabetes. In general, every percentage point drop in A1C blood test results (e.g., from 8.0% to 7.0%) reduces the risk of microvascular complications (eye, kidney, and nerve diseases) by 40%.

- Blood pressure control reduces the risk of cardiovascular disease (heart disease or stroke) among persons with diabetes by 33% to 50%, and the risk of microvascular complications (eye, kidney, and nerve diseases) by approximately 33%.
- In general, for every 10 mm Hg reduction in systolic blood pressure, the risk for any complication related to diabetes is reduced by 12%.
- Improved control of cholesterol or blood lipids (for example, HDL, LDL, and triglycerides) can reduce cardiovascular complications by 20% to 50%.

As kidney disease, heart disease, blindness, retinopathy, glaucoma, amputation, abdominal pain, nerve damage, erectile dysfunction, hypertension, loss of sensation or heightened pain sensation, and gastroparesis are common complications of diabetes, we find ample documentation to satisfy the requirement that diabetes be debilitating. Furthermore, the documentation citing diabetes as a disease characterized by chronic vomiting and nausea, as well as severe abdominal pain, and in some circumstances cachexia, demonstrates it as a disease that already satisfies three symptoms that medical marijuana use is legally permitted for. In addition, our discussions will demonstrate that marijuana can lower blood pressure, improve sexual physiology, prevent microangiopathies, and restore normal insulin signaling.

IV. Scientific Evidence Supporting Use of Medical Marijuana for Diabetes Mellitus

Control of glucose levels has unanimously been demonstrated as the best means to prevent secondary complications of diabetes mellitus. Thus, pharmaceutical agents that can sensitize the body to insulin signaling, normalize insulin secretion, reduce agents that inhibit insulin signaling, or inhibit toxic by-products of hyperglycemia, are the most beneficial treatments for the diabetic state. As we shall demonstrate, the use of marijuana has therapeutic benefits at every level previously mentioned. Before this discussion, a basic review of insulin signaling is required.

Upon binding to the heterotetrameric insulin receptor (IR), insulin causes tyrosine autophosphorylation of the receptor's β-subunits[16], which consequently results in dissociation of the activated insulin receptor substrate-1 (IRS-1)[17]. IRS-1 also retains sites for tyrosine phosphorylation that become occupied during IR autophosphorylation[18]. Once dissociated from the IR receptor, IRS-1 transfers its P_i to PI3K [19], a process that is responsible for the translocation of GLUT-4 to the plasma membrane. The IRS-2 is similarly responsible for the activation of PI3K, however, only activates after prolonged IR tyrosine autophosphorylation and requires a higher cytosolic concentration[20]. In states of excess insulin activity at the IR, autophosphorylation of both serine and threonine sites can occur as a means to down regulate activation of PI3K via IRS-1 or IRS-2[21] [22]. In addition to inhibitory phosphorylation sites as a down-regulatory mechanism to

PI3K activation, both IRS-3 and IRS-4 will inhibit PI3K-mediated GLUT-4 translocation[23]. In a separate cascade, autophosphorylation of the IR is responsible for the Ras/Raf initiated activation of ERK1/2, also know as MAPKp42/p44. Ultimately, this sequence is an amplification mechanism to activate various transcription factors and nuclear receptors. Most notable of these nuclear proteins is the PPARγ receptor, which is responsible for a multitude of IR mediated glucose and lipid metabolizing effects. The most utilized class of pharmaceuticals in the treatment of Type II diabetes, thiazolidinediones (TZDs) exert their insulin sensitizing influence by binding with high affinity to the PPARγ. The PPARγ is a class II nuclear receptor, meaning it will dimerize with the retinoic X receptor. Activation of both PPARγ and retinoic X receptors has been demonstrated to lower glucose plasma levels[24] [25]. In addition, PPARγ activity can inhibit gene transcription of TNFα, plasminogen activator-inhibitor-1, leptin, resistin, and interleukins -6 and -11, all of which have been found to increase insulin resistance[26]. Furthermore, transcription of insulin-IR sensitivity enhancing proteins adiponectin, fatty acid transport protein, and IRS-2, are all up-regulated by PPARγ activation.

1. THC Reduces Progression of Diabetes in Rodent Models:

The streptotozocin (STZ) virus is administered to rodents being utilized in studies investigating pharmacological actions of antidiabetic agents in models of Type 1 DM. When given at higher doses (200mg/kg), STZ employs a rapid cytotoxic response against β-cells, which uniquely express the glutamic acid decarboxylase enzyme[27]. Employing gradual doses of STZ (5-40mg), researchers designed a mouse model of Type 1 DM that parallels that of humans in that hyperglycemia is slow in onset, with gradual progression of destruction to the pancreas resulting from lymphocytic infiltration[28]. Mammalian immunological studies on Type 1 DM find an imbalance in the TH1/TH2 profile, with up-regulation of TH1 and its characteristic cytokines[29] [30]. When THC was given gradually to STZ infected mice, mRNA levels of the TH1 cytokines TNFα, IL-12, and IFN-γ were significantly decreased[27]. THC was also responsible for slowing the progression of elevated serum glucose and inhibiting the loss of insulin secretion, as compared to controls. As we shall see in the next section, the inflammatory response in conjunction with hyperlipidemia, are the primary causal factors in hyperglycemia, resulting in nearly all secondary complications of the diabetic state.

2. Lipids, Inflammatory Cytokines, and Diabetes:

While there are differences in insulin resistance mechanisms between muscle and adipose tissue, it is apparent the underlying pathology causing improper IR signaling is due to both an inflammatory and hyperlipidemic state. Increased concentrations of both FFAs in muscle tissue and their respective metabolites, long chain acyl-CoA, diacylglcerol (DAG), and triglycerides, have been correlated with decreased insulin signaling in the rat[31]. Insulin resistance

due to elevated plasma FFA levels follows homologous mechanisms between humans and other mammals[32 33 34 35 36]. More specifically, prolonged lipid exposure initiates insulin resistance in both fast- and slow-twitch muscle fibers studied in vivo from both human and rat studies[37 38 39]. Increased FFAs inhibit insulin mediated glucose disposal mechanisms of both oxidative and non-oxidative origins[40]. In both humans and rats, infusion of FFAs or high fat diets are associated with the insulin resistant state[41 42 43].

As previously mentioned, the IR cascade involves tyrosine autophosphorylation. DAG, TAG, and other by-products of FFA metabolism have been shown to activate the intercellular stress kinases JNK and PKCθ, both of which activate serine residues located on the IRS-1[44 45 46 47 48 49]. A study was conducted on human 3T3-L1 adipocytes utilizing various mixtures of both saturated & unsaturated FFAs at physiologic concentrations[50]. Lysates were compared to controls via several immunoblotting techniques and SDS-PAGE. In this study, FFAs inhibited IRβ tyrosine autophosphorylation and caused a 40% decrease in IRβ expression levels. Near homologous data was found for FFA activities against IRS-1. Similar inhibitory effects on AKT/PKB were found, however, no changes in total protein levels were observed. Other studies also identify decreased PKB phosphorylation in rat soleus muscle[51], as well as inhibited IRS-1 & -2 mediated PI3K and AKT activation during in vivo FA infusion studies in mammals[52 53]. An assay has been developed to identify inhibitors of GLUT-4 translocation[54]. Treatment of 3T3-L1 adipocytes with FFAs at 500μM for 3 hours resulted in complete inhibition of GLUT-4 translocation. Furthermore, FFA treatment at 1mM for 1 hour, .5mM for 3 hours, and .3mM for 6 hours, results in a 70-90% inhibition of IR mediated glucose uptake initiated by 1.7mM of insulin.

While FFA accumulation is found to cause insulin resistance via activation of PKCθ and its consequent serine phosphorylation of IRS-1 & -2, the most profound effects on insulin mediated glucose metabolism are seen from inflammatory cytokines. A high correlation between elevated FFAs and TNFα have been found in the diabetic state[55]. TNFα of adipose origin is increased in both humans and rodents in obesity related insulin resistant states[56 57]. Increased mRNA expression levels of TNFα within adipose tissue are directly correlated to hyperinsulinemia[58]. This inflammatory cytokine is elevated to 2.5x normal concentration in obesity[59], and is directly linked to a multitude of pathological mechanisms underlying insulin resistance[60]. The link between insulin resistance, elevated FFAs, and increased inflammatory cytokines is an up-regulating process. Both TNFα and FFAs increase JNK expression[61]. JNK itself can cause insulin desensitization, as it initiates direct phosphorylation of ser307 of IRS-1[62]. In both diet-induced and genetic rodent models of obesity, pharmacologically induced JNK deficiency enhanced insulin signaling and sensitivity[63]. Hepatic JNK suppression inhibits regulatory enzymes of gluconeogenesis[64]. JNK and IKKβ phosphorylation are exponentially increased during FFA treatments[49]. Upregulation of TNFα gene expression is the result of

increased JNK phosphorylation[65]. ELISA analysis revealed that TNFα secretion increases 80% with FFA treatment in 3T3-L1 adipocytes[49]. The same group also found restored glucose metabolism in JNK adipocyte knockouts treated with the same levels of FFAs. TNFα in turn, has been demonstrated to elevate plasma FFAs[66 67]. This phenomenon is in part due to lipolysis. TNFα decreases perilipin levels[68]. Perilipin inhibits hormone sensitive lipase (HSL) adhesion to fat droplet surfaces, where HSL is responsible for lipid metabolism. TNFα induced lipolysis is known to occur in rat, mouse, and human adipocytes[69 70 71 72]. Thus, we see a cyclic upregulation of the hyperlipidemic-pro-inflammatory cytokine state in insulin resistance.

TNFα is by far the most inhibiting factor of insulin mediated glucose metabolism both in vivo and in vitro[73 74], in animal models[75 76 77], and in humans[78]. At a macroscopic level, TNFα inhibits both peripheral glucose uptake as well as insulin mediated suppression of hepatic gluconeogenesis[79]. TNFα also regulates several important cytokines in insulin resistance. Of most importance is the TNFα mediated inhibition of adiponectin secretion[80] and gene expression levels in both immature and fully differentiated 3T3-L1 adipocytes[81] . Adiponectin is a skeletal muscle tissue insulin sensitizing adipokine found in both humans and rodents. TNFα also causes increases in insulin desensitizing cytokines. Suppressor of cytokine signaling -1 & -3 (SOCS-1/-3) are known to mediate several aspects of TNFα induced insulin resistance[82 83 84]. In white adipose tissue, SOCS-3 levels are increased over a prolonged duration as a result of elevated TNFα[82]. SOCS-1 & -3 are known to cause IRS-1 &-2 breakdown via the ubiquitin pathway[85]. Both tyrosine phosphorylation of IRS-1 and its ability to activate PI3K are inhibited by elevated levels of SOCS-3 in a COS-7 cell line[82]. SOCS-1 &-3 are both responsible for elevated FA synthesis via activation of the SREBP-1c transcription factor[86].

TNFα is also responsible for direct actions on IR signaling. It has been shown to decrease both IRS and GLUT-4 protein levels[87]. Inhibition of autophosphorylation within the IR is seen in studies employing both “low dose chronic”[88] and “short term incubation”[89] of adipocytes with TNFα. This inflammatory cytokine is known to inhibit tyrosine phosphorylation sites of the IR, all the IRS proteins, and protein phosphatase-1[90 91]. In human adipocytes, rat hepatocytes, human fibroblast NIH-3T3 cells, and embryonic human 293 kidney cells, TNFα is shown to decrease tyrosine phosphorylation of both IR and IRS-1[92 93 94]. Whether by serine phosphatase inhibition, serine kinase activation, or a combination of both, it has been demonstrated that TNFα induces serine phosphorylation of IRS-1 with devastating results to glucose metabolism[95]. AN example of this is the IRS-1 conversion into an IR tyrosine kinase inhibitor[96]. In addition, these serine activated IRS-1 proteins are extremely unstable and quickly degrade[97].

TNFα causes changes in adipocyte differentiation, gene expression, and protein levels that ultimately result in improper lipid and glucose metabolism.

The fully differentiated 3T3-L1 human adipocyte expresses both Glut-1 and -4, however, only Glut-1 is transcribed in preadipocytes[98] [99]. TNFα prevents preadipocyte development in both 3T3-L1 cells and other preadipocytes[100] [101] [102]. TNFα treatment (.04nmol/l/24h) reduced ACRP30 gene expression by 27%[103]. ACRP30 is a well documented adipokine that inhibits hepatic gluconeogenesis, enhances skeletal muscle FA oxidation, and interestingly, enhances weight loss without an anorexigenic response[104] [105]. The same study utilized oligonucleotide microarray analysis of 3T3-L1 human adipocytes following TNFα treatment. Downregulation occurred at rates of -3.4x for CEBPα, -2.3x for RXRα, and -2.0x for PPARγ. CEBPα restricts growth arrest in mature adipocytes and facilitates metabolism[106] where it is highly expressed and its isoform, CEBPβ is suppressed[107]. CEBPβ expression was increased 1.6x during TNFα treatment. Dimerization of CEBPβ and NFκβ, a well known central mediator to a multitude of inflammatory pathways, is known to occur and increase gene expression(Hotam, 1995). Other significant protein expression alterations included an 8-fold increase in iIKK, the rate limiting protein for Iκβ degradation[108]. Taken in combination with the discovery that TNFα facilitates NFκβ translocation from the cytoplasm to the nucleus, we see yet another mechanism by which TNFα induces a broader-range inflammatory response.

TNFα also inhibits metabolism and insulin signaling in skeletal muscle. Skeletal muscle constitutes the major target tissue for Type 2 DM insulin resistance[109]. FA and glucose metabolism in skeletal muscle is tightly regulated by AMP kinase[110]. AMPK is an enzyme that phosphorylates acetylCoA carboxylase (ACC), which enhances FA oxidation in skeletal muscle[111] [112]. AMPK activity has also been correlated with increased mitochondrial biogenesis, an important factor in FA oxidation[113]. Activating AMPK with the agonist AICAR has been demonstrated to facilitate glucose metabolism in a Wortmannin (PI3K antagonist) inhibiting fashion[114] [115] [116]. AMPK activity is allosterically enhanced with an increase in the AMP: ATP levels[117] [118] [119], while protein phosphatase-2 (PP2-C) is known to mediate AMPK dephosphorylation[120]. Exercise has also been demonstrated to enhance AMPK activity[121] [122] [123]. This effect is attributed to the insulin sensitizing effects of exercise observed in animals and humans with Type 2 DM[124] [125]. TNFα causes both de-activation of AMPK via a 27% reduction in ACC phosphorylation, as well as causing an up-regulation of PP2-C and decreased Thr172 phosphorylation within the active site of AMPK[126].

Besides TNFα underlying a multitude of insulin desensitizing mechanisms, other inflammatory adipokines/cytokines have been found to cause additional metabolic deficiencies in the diabetic state. Interleukin-6 (IL-6) has been found to cause insulin resistance through several actions in humans[127]. IL-6 is a proinflammatory adipokine found elevated in states of glucose intolerance, obesity, and Type 2 diabetes[128] [129] [130] [131] [132] [133]. Increased levels of IL-6 have been correlated with risk of developing Type 2 DM[134]. Utilizing RT-PCR and various immunoprecipitating techniques, it has been demonstrated that IL-6 reduces expression of IRS-1 and GLUT-4 by 35%, as well as decreased both

GLUT-4 and PPARγ mRNA levels[135]. The same group also identified an up-regulation mechanism of IL-6 by TNFα, both of which regulate the JAK-STAT pathway. Both IL-6 and TNFα inhibit IRS-1 tyrosine phosphorylation via preferential ser307 phosphorylation mediated by the activation of SOCS-3[136 137]. Furthermore, a second group identified an inhibitory effect against Akt and PI3K activation, in addition to IRS-1 inactivation[138]. In both rodent and human 3T3-L1 adipocytes, IL-6 inhibits lipoprotein lipase (LPL) activation[139]. Enhanced activation of LPL has been demonstrated to relieve insulin resistance[140 141]. This enzyme regulates the rate limiting step of hydrolyzing lipoproteins abundant in triglycerides[142].

3. Cannabinoids Enhance FFA & Glucose Metabolism by Reducing the Pro-Inflammatory State.

Cannabinoids can enhance FFA and glucose metabolism via activation of PPARγ[143 144 145]. Reversal of the diabetic state has been correlated to PPARγ activation[146]. Like cannabinoids, oxyiminoacetic acid derivatives and the previously mentioned TZDs are pharmaceutical agents that exert an antidiabetic activity via activation of PPARγ[147 148 149 150]. The most widely distributed antidiabetic drugs in the world are TZDs[151 152]. Common side effects of TZDs include both weight gain and toxicity[148]. This weight gain may in fact reduce FFAs by incorporation into adipocytes undergoing differentiation. PPARγ facilitates early differentiation of fibroblasts into preadipocytes[153], adipogenesis[154], and late stage maturation[155]. As a transcription factor, PPARγ enhances FFA metabolism by upregulating FABP, LPL, acyl-CoA synthase, adiponectin, fatty acid transport protein (FATP), and IRS-2[156 157]. In addition, PPARγ inhibits transcription of the insulin desensitizing factors TNFα, PAI-1, resistan, leptin, and IL-6 & -11[26].

Within the brain, cannabinoids can also increase metabolism of glucose and FFAs via activation of the AMPK enzyme[158] that, as previously discussed, is inhibited by inflammatory cytokines and FFAs. This in turn may prevent hyperglycemic pathology in the CNS. Cannabinoid inhibition of inflammatory mediators may also enhance the activity of AMPK in peripheral tissues, although no studies on the diabetic state and treatment with cannabis have focused on AMPK activation.

Cannabinoids also impart a potent anti-inflammatory response characterized by inhibition of various cytokine pathways. We previously discussed the shift in the Th1/Th2 ratio, with an increase in Th1 cells and their respective inflammatory cytokines in the diabetic state. Cannabinoid treatment under variable experimental conditions has been found to bring the Th1/Th2 profile into equilibrium, and in other circumstances, to increase Th2 and decrease Th1[159 160 161 162]. Inflammatory cytokines involved in insulin resistance of multiple origins have unanimously been demonstrated to be down regulated by THC. THC decreases TNFα production or expression from human and mouse

macrophage cell lineages[163], human in vitro NK cells[164 165], and with CBD in peripheral blood mononuclear cells[166]. Cannabinoids have also been shown to inhibit IL-1, -6, -10, and -12[162 167].

4. Cannabis, Inflammation, VEGF, and Retinopathy

In addition to inflammatory cytokines being elevated in the diabetic state, COX-1 and -2 synthesized inflammatory mediators are produced in excess from arachidonic acid[168 169]. Indeed, several thromboxanes, prostacyclins, and prostaglandins of COX origin have been demonstrated to induce ocular inflammation that underlies the pathological progression of diabetic retinopathy, both by itself and in its ability to up-regulate vascular endothelial growth factor (VEGF)[170 171 172 173 174]. Inflammatory prostanoids are known to be synthesized by COX-2 under elevated glucose conditions[175 176]. Even in the newly diagnosed diabetic, alterations in hyperpermeability are visible, and considered to be mediated by both VEGF and TNFα[177 178 179]. VEGF has an established role in increasing expression of intercellular adhesion molecule-1 (ICAM-1), which exacerbates cytokine secretion via leukocyte activation[219]. ICAM-1 expression also increases in the earliest stages of diabetes due to oxidative stress mediated by hyperglycemia[169 219]. Increases in ICAM-1 levels in diabetics are related to neural apoptosis during ischemic conditions[180]. As much as a 3-fold elevation in ICAM-1 levels were observed in diabetic retinal tissue compared to controls[181]. VEGF protein levels are increased during hyperglycemia and in this elevated state, increase COX-mediated inflammatory prostacyclin synthesis via induction of STAT3[182 183 184 185]. STAT3 is a common intercellular kinase that is activated by oxidative stress from free radicals, TNFα, and other mediators of inflammation[186]. Cellular damage via oxidative stress mediated upregulation of VEGF is considered one of the primary pathological mechanisms underlying diabetic retinopathy[187 188 189 190]. Reactive oxygen species (ROS) are synthesized via the mitochondrial electron transport chain and NADPH oxidase under hyperglycemic conditions[191 192]. Another point of self-perpetuating upregulation between diabetic retinal pathological systems involves the p38MAPK protein. p38 phosphorylation is associated with diabetic vascular hyperpermeability, retinal ganglion apoptosis, NMDA mediated neural cell death, and is activated by hyperglycemia, pro-inflammatory cytokines, and VEGF[193 194 195 196 197]. Thus we see a complete up-regulating system between free radicals, inflammatory cytokines, VEGF, and the pro-inflammatory COX derived metabolites.

Between these pathological systems, VEGF is considered the central mediator of retinopathy[198 199]. Furthermore, the mechanisms of retinopathy are considered homologous between rat STZ models and human subjects, as both are mediated by VEGF and VEGFR2 upregulation[200 201]. Rat, mouse, and human models of diabetic retinopathy identify neuronal and glial apoptosis, even in the earliest stages of the disease[202 203 204 205]. In addition, both Type 1 and 2

DM induced retinopathy is characterized by similar biochemical and microvascular alterations[183].

The progression of diabetic retinopathy can be attributed to 4 main pathological processes[10 11 206], which include microaneurysms, increased vascular permeability, capillary occlusion, and neovascular proliferation. The blood-retinal barrier, composed of pericytes and endothelial cells, often becomes damaged in the diabetic pro-inflammatory and hyperglycemic state[207]. As pericytes are the main nutritional source for retinal endothelial cells, the blood-retinal barrier's protective functions become impaired and can lead to capillary leakage[208]. In the more advanced stages, this pathology can give rise to macular edema and complete blindness[209]. Diabetic induced retinal hyperpermeability is directly proportional to the elevation in VEGF expression[177 210 211 212]. VEGF induces NOs activation resulting in prolonged hyperpermeability and down-regulation of the occluding protein, found on tight junctions[213 214 215]. Hyperglycemia is known to cause capillary occlusion, which subsequently creates pockets of retinal ischemia and hypoxia[216]. These two conditions set the stage for angiogenesis primarily mediated by VEGF. Neovascularization occurs to return blood flow to ischemic areas[217]. VEGF synthesized by the vascular smooth muscle layer, pigmented epithelium cells, pericytes, and neural retina, as well as its receptor VEGFR2 on epithelial cells, are both upregulated after retinal ischemia[179 218 219 220 221 222]. Differentiation and proliferation of endothelial cells is mediated by VEGF, resulting in the formation of new capillaries[223].

VEGF mRNA stability and expression are enhanced under hypoxic conditions[224]. Hyperglycemia not only up-regulates VEGF via hypoxic conditions, but increases VEGF through increasing TNFα, IGF-1, advanced glycation end products (AGEs), ROS, and multiple ILs of which are all elevated in the diabetic condition[225 226 227 228 229 230]. The free radicals NO, superoxide anion, and peroxide are quickly produced upon VEGF-VEGFR2 binding and are incorporated into the activation of VEGFs mitogenic cascade[225 231 232 233]. As with many of the other mechanisms discussed, the relationship between AGEs, ROS, oxidative stress, and inflammatory regulators in the retina, is an up-regulating system. The polyol pathway is well established in diabetics. Occurring primarily in tissues non-responsive to insulin mediated glucose uptake, exhausted glycolytic enzymes reduce activity, followed by increases in sorbitol and fructose accumulation via the activity of aldose reductase and sorbitol dehydrogenase[234]. Elevated levels of sorbitol and fructose then elevate the $NADH/NAD^+$ ratio[235 236 237], resulting in a hypoxic condition[235]. In addition to oxidative stress, the polyol pathway becomes activated in response to prostaglandin synthesis and COX activity[238 239 240]. Hyperglycemia also stimulates phosphorylation of the DAG-PKC pathway[241], whose activation is also increased by an elevation in the $NADH/NAD^+$ ratio[235 242]. PKC activation and resulting DAG accumulation are well established as mediators enhancing the secretion of many growth factors involved in angiogensis[237 241 243 244]. Free radicals in the form of hydroxyl anions accumulate in the diabetic condition as a

result of glucose auto oxidation in ketoacidotic environments[245]. We previously mentioned the VEGF induced NO accumulation as a means of increasing hyperpermeability; here we review other mechanisms leading to NO production in both retinopathy and glaucoma. Glutamatergic transmission occurs in photoreceptor, bipolar, and ganglion cells of the retina (Adamis, 1994). Increased glutamate secretion occurs in response to capillary occlusion. Over-activation of the glutamate-NMDA receptor results in an accumulation of intracellular Ca^{2+}, which in turn activates the NOs enzyme(Takeda, 2001)[246 247]. Excess NO production favors synthesis of peroxynitrites from free radical anions, which impart an exponentially greater response of cellular damage[248] than anions alone. Retinal ischemic injury is directly associated with increases in metabolites from NO, superoxides, and peroxynitrite[249]. Above normal physiological concentrations of free radicals can favor phosphorylation of PKC, increase sorbitol levels, translocate NF-κβ, and favor the non-enzymatic formations of AGEs[250]. The non-enzymatic covalent binding of glucose to long-lived proteins and lipids under hyperglycemic conditions creates AGEs[251]. AGEs in turn, are known to promote pro-inflammatory cytokine secretion and NF-κβ activation[252 253 254 255].

This entire up-regulatory pathology of retinopathy can be alleviated by administration of whole-Cannabis plant material. Besides the anti-inflammatory effects of cannabinoids, non-cannabinoid derived products from the marijuana plant have proven successful in reducing COX derived metabolites that are elevated in the diabetic condition. Cannflavin is a prenylated flavone antioxidant unique to the marijuana plant. Cannflavin has 30x more potent of a COX inhibitory effect than aspirin[256 257]. General constituents of the volatile fraction of a Cannabis extract have demonstrated anti-inflammatory properties via COX inhibition[258 259]. The carageenan induced paw edema assay is used to study a multitude of inflammatory pathways in rodents. In this design, THC has been tested to have twice the anti-inflammatory strength as hydrocortisone, and more profoundly, 80x the potency of aspirin[260 261]. Utilizing the same model, other groups identified the cannabinoid CBC to have quite a potent anti-inflammatory characteristic[262 263]. The pyrrolized metabolites of CBD also demonstrate a substantial decrease in COX-1 activation[264].

Cannabinoids have profound inhibitory effects on angiogenesis via inhibition of the VEGF pathway[265 266]. CBD inhibits ICAM-1 expression[181], thereby reducing the inflammatory cytokine response, neural apoptosis, and retinal ischemia[180] observed in retinopathy. CBD also reduces TNFα in retinal tissue, as determined using a sandwich ELISA[181]. p38 activation is markedly inhibited following CBD treatment, as well as almost complete inhibition of tyrosine nitration[181] . The same group found CBD to also prevent hyperpermeability and cell death. Both in vitro and in vivo studies identify antioxidants as therapeutic agents to prevent glutamatergic/NMDA induced neurotoxicity resultant from ischemia[267 268]. THC, CBD, and Win55,212 antagonize glutamatergic neurotoxicity via a CB1 dependent mechanism in

individual neurons in vitro as well as in whole brain studies[269 270 271]. THC, CBD, and the synthetic agonist HU-210 all behave as potent antioxidants, protecting against free radical and glutamatergic cell death[272 273]. THC not only prevents blood-retinal-barrier degradation, but also restores the damaged tissue to original thickness[274], thereby inhibiting vascular hyperpermeability. Both THC and CBD (.4 & 2mg/kg) inhibit neuronal apoptosis and NMDA mediated tyrosine nitration induced by NMDA (200nmol/eye)[274], as determined by thickness of retina in combination with a TUNEL assay. The same research group confirmed these findings using an immunohistochemical slot blot technique in addition to immunofluorescence of nitrated tyrosine residues. The authors went further to identify specifically how THC inhibits destruction of retinal neural conduction. Utilizing RT-PCR, retinal ganglion cell mass was measured with the Thy-1 antigen, and ganglion axon with the NF-L marker[275 276]. After discovering that elevations in NMDA promoted loss of both Thy-1 and NF-L, the group found that THC inhibits breakdown of both these structures in a dose-dependant fashion. THC and CBD both offer unique therapeutic benefits. CBD increases the stability of the endocannabinoid anandamide, a well documented, potent neuroprotectant acting at a multitude of receptor and non-receptor mediated mechanisms[277 278 279 280]. CB1 activation by THC promotes phosphorylation of neuroprotective MAPK pathways[281]. THC also inhibits production of nitrite and nitrate free radicals in the retina[274]. In addition to these beneficial effects for both retinopathy and glaucoma, both animal and human studies identify the CB1 agonists THC, Win55,212, 2-AG, and HU-211 to alleviate the intraocular eye pressure associated with hyperpermeability[282 283 284 285 286].

5. Cannabinoids and Atherosclerosis:

Atherosclerosis is a common disease occurring in diabetics[287 288]. This is likely due to similar chronic inflammatory and adhesion protein pathological states. Severe clinical complications can occur acutely upon thrombosis and plaque rupture[289 290]. Elevated levels of ICAM-1, VCAM-1, and E-selectin have been identified in the development of atherosclerosis, Type 1, and Type 2 diabetes mellitus, in addition to being correlated to both hyperinsulinemia and hyperglycemia [291 292 293 294 295 296 297]. Advancement of Atherosclerosis results from imbalances between the Th1/Th2 cytokine profile[298], due to endothelial injury. As with the imbalance observed in diabetes, there is an up-regulation of Th1 inflammatory cytokines seen in early and advanced atherosclerotic lesions[299 300]. The ApoE-/- mouse model of Atherosclerosis mimics human pathology of this disease[301]. Their hypocholesteremic condition results in fat accumulation within vessel walls, with visible lesions occurring by the 5th week of induced high fat diet.

In both human and rodents, immunohistochemical techniques reveal the distribution of CB2 receptors within atherosclerotic lesions, whereas no staining occurs within healthy arteries[298 302] . CBD was discovered to block macrophage chemotaxis, an early stage of atherosclerotic lesion formation, in a CB2

dependent fashion, both in vivo and in vitro[303] . Also found to be CB2 dependent was THC's ability to block leukocyte adhesion in mouse models of atherosclerosis[298]. THC has been found in vitro to inhibit macrophage chemotaxis resulting from MCP-1 activation, as well as down-regulating chemokine receptor CCR2. Both effects were blocked by the CB2 antagonist SR144528[304] . Both IFNγ of lymphoid origin and macrophage infiltration of developed lesions is decreased with THC treatment [299] . Both the CB2 selective agonist JWH and the endocannabinoid anandamide inhibit CD8 T-lymphocyte chemotaxis resulting from CXCL12 receptor activation[305].

Utilizing the ApoE-/- atherosclerosis model[298], as well as in vivo[306 307], THC, CBD, and anandamide have all been found to attenuate advancement of atherosclerotic lesion formation. Within 11 weeks of a high fat diet in ApoE-/- mice, atherosclerotic lesions are visible throughout aortic roots. THC treatment in this model demonstrated similar arterial composition to controls[298]. Other beneficial effects of THC in atherosclerotic development that were abolished by SR144528 included reduced macrophage infiltration, leukocyte adhesion, MCP-1 induced leukocyte migration, and decreased TNFα stimulated CCR2 up-regulation.

Attenuation of atherosclerotic pathology of cannabinoids lies once again in their ability to bind to PPARγ. PPARγ agonists have been demonstrated to increase lipid storage while simultaneously inhibit cytokine stimulated macrophage activation[308 309 310 311]. The production of inflammatory mediators in T-cells, endothelial cells, and smooth muscle cells, has been found to be inhibited by PPARγ ligands[312 313]. Given these beneficial preventative effects of phytocannabinoids, in combination with the ability of THC to shift the T-cell balance towards a Th2/Th1 profile[165 314] in humans, we find ample evidence demonstrating cannabis may have a beneficial effect in diabetics by reducing development and progression of atherosclerotic lesions.

6. Cannabis and Cardiovascular Complications of Diabetes:

At the beginning of this presentation we discussed the high incidence of heart disease and stroke, as well as the mortality rate of diabetics due to hypertension. We had also mentioned conditions of hypoxia due to hyperglycemia. Here we shall now discuss the beneficial effects of marijuana in the diabetic state with regards to cardiovascular complications.

The endocannabinoid anandamide is responsible for vasodilation, mediated at the transient receptor potential vanilloid receptor (TRPV-1)[315]. Importance of cardiovascular effects of endocannabinoids in consideration of phytocannabinoid treatment lies in the discovery that CBD can inhibit both enzymatic degredation by anandamide amidase as well as block anandamide reuptake[316 317]. Repeated treatments with THC cause down-regulation of sympathetic nervous system activity and increased parasympathetic activity

resulting in brachycardia and lowered blood pressure in humans[318]. Animal models also display hypotension and brachycardia[319]. The vasorelaxant effects of THC are mediated by PPARγ[320]. PPARγ activators have been correlated to vasorelaxation, elevated NO bioavailability, blood pressure decreases, and reduced atherosclerotic development[321 322]. In a rodent model, THC (10μM) and the TZD, rosiglitazone (30μM) followed homologous cardiovascular effects[320]. In this study, CB1 antagonists were found ineffective at inhibiting these effects, while a PPARγ inhibitor completely blocked vasodilation. THC was also found to elevate levels of the same prostanoids associated with the vasodilatory effects of TZDs[323] .

The CB1 agonist HU-211 displays an elevated attenuation to epinephrine's arrythmogenic effects during arterial occlusion and reperfusion in rodent models[324]. Ischemic conditions result from various types of arterial and cerebral occlusions in diabetes resulting from hyperglycemia. Under animal models of ischemia-induced brain damage, therapies inducing hypothermia prove most efficacious[325]. THC has proven to be effective at induction of hypothermia[326]. Both THC and CBD attenuate the oxidative potential during an infarction as assessed by cyclic voltammetry[327]. THC and CBD also decrease the volume of infarction resulting from cerebral capillary occlusion in rodents[328]. Both the hypothermic and infarction effects of these cannabinoids were completely abolished by the CB1 antagonist SR141716.

THC can protect the heart from hypoxia. Using cardiomyocytes under hypoxic conditions with no glucose, THC had a maximal reduction of lactate dehydrogenase release, an indicator of oxidative stress, from 388% to 129% normal concentrations in controls[329]. Interestingly, LDH release was not affected by THC under normal oxygen conditions. The effect was found to be inhibited by the CB2 selective antagonist SR144528. It has long been known the cardioprotective effects of pre-conditioning induced NO production against hypoxic cellular stress[330]. iNOS-mediated NO production has beneficial vasodilatory effects in heart cells in vivo[331 332]. THC mediated elevations in NO by cardiac cells was found to be CB2 dependent[322]. This same group found that hypoxia reduces cardiac fiber density. THC treatments resulted in indistinguishable fibers between THC treated tissues and controls.

It should be clarified that localized production of NO can have both beneficial and negative consequences in diabetes. Various knockout studies identify nervous system localized nNOS and iNOS to increase neural damage caused by cerebral ischemia and arterial occlusion, and eNOS to prevent injury from ischemia[333]. CB1 activation on cerebellar granule cells blocks membrane depolarization and inhibits nNOS Ca^{2+} -dependent NO production[334]. nNOS and CB1 co-localization has been demonstrated with high homology throughout the nervous system with similar results being reported[335]. The vasodilatory effect of anandamide is conferred via NO production by eNOS, as demonstrated by blocking with L-NAME[336]. The soluble adhesion molecules MCP-1, ICAM-1, and

VCAM-1, as well as platelet aggregation, are all inhibited by eNOS[337]. Indeed, hyperglycemia has an inhibitory effect on endothelial vasodilation[338 339]. Additionally, TNFα is known to activate iNOS[340] in the nervous system, leading to such conditions as hyperalgesia.

7. Cannabis, Neuropathies, Excitoxicity, and ROS:

Neurodegeneration is a fundamental process in retinopathy[203]. Ganglion and retinal cell death occur from neurotoxicity[181]. Neural loss and injury have been implicated in diabetes from the brain to the periphery[341]. Other neurologic complications of diabetes observed in humans and rodents include dementia, learning deficits, Alzheimer's, decreased abilities on neuropsychological tests, and an extremely high incidence of depression[342 343 344 345 346 347 348 349 350 351].

Glutamatergic excitotoxicity is a well documented pathological state leading to various neuropathies in diabetes[352]. Specifically, hyperglycemic-induced ischemia activates glutamatergic-excitotoxic apoptosis[353 354 355 356]. Neurotoxicity via glutamatergic signaling occurs through several mechanisms at the NMDA receptor. Associated with excessive activation of the NMDA receptor is an elevation in intracellular Ca^{2+}, which subsequently generates mitochondrial and apoptotic reactive oxygen species (ROS)[357 358]. Apoptotic events are associated with diabetic neurological, retinal, endothelial, and kidney complications[247 359 360]. Nervous system cells known to be damaged and signaled to apoptosis under hyperosmolar conditions include Schwann cells, neuroblastoma, dorsal root ganglion, and hippocampal neural circuits[361 362]. Apoptosis is also a fundamental phenomenon occurring in STZ induced experimental rodent models of diabetes[363 364 365]. Cell death can be initiated by increases in intracellular Ca^{2+}, which in turn signal cytoskeletal degredation, impaired energy expenditure, and activate hydrolytic enzymes[366]. Inflammatory mediators are also known to play a pivotal role in diabetic neurodegenerative pathology. ERK, JNK, and p38 have all been found to be elevated during NO and NMDA induced apoptotic events[367 368 369 370]. Axonal degeneration is a well established feature of increased JNK phosphorylation[371 372]. IGF-1 has been shown to exert an anti-inflammatory/neuroprotective, bi-directional regulatory control over JKK and p38 phosphorylation states[373 374 375 376 377]. These studies additionally identify oxidative stress as the means by which NF-Kβ becomes activated and leads to axonal death. Improper IGF-1 signaling and decreased levels have been identified in Type 1 diabetics. Neuronal damage has been linked to up-regulation of nNOS activity and COX metabolites[378]. Hyperglycemia itself directly causes increased superoxide production, mitochondrial dysregulation, and stresses of oxidative and nitrosative origin to the nervous system[379].

Further means of neural degeneration in diabetes occur by decreased neurotrophic and antioxidant activities[380 381 382]. The anti-apoptotic and neuroprotective effects of both C-reactive peptide (CRP) and insulin are well

characterized[383 384]. CRP treatment benefits patients by enhancing autonomic nerve conduction and metabolism, as well as increasing neuro-regeneration[385 386]. Similar to cannabinoids, CRP activates eNOS, thereby enhancing vascular flow[387]. Neurotrophic growth factor (NGF) is impaired in STZ diabetes induced rodents and exerts trophic signaling for small-sensory neuron homeostasis[388 389 390 391]. Bradykinin and H-ion potentiated inflammatory nociception is further enhanced by NGF, in addition to up-regulating secretion of hyperalgesic neurotransmitters[392 393 394 395]. Taurine functions as an osmolyte, neurotrophic factor, and antioxidant; its levels have been shown to be decreased in the diabetic state[396 397]. Hyperglycemia elevates fructose and sorbitol intracellular concentrations resulting in depletion of osmolytes, including taurine[398].

The results of these various oxidative and inflammatory attacks on the nervous system reflect the unique types of analgesia seen in the diabetic state in addition to diabetic neuropathies. Two common symptoms of diabetes include thermal hyperalgesia and mechanical allodynia[399 400 401 402 403], both in humans and rodent models. Diabetic neuropathies are often one of the most difficult forms of analgesia to treat[404 405 406 407]. Opiates prove limited in efficacy under both clinical trials and experimental animal models[408 409 410 411].

Neuropathic pain has been found to be mediated by TNFα, IL-1, and IL-6[412]. TNFα can both activate nociceptive transmission and induce hyperalgesia[413 414]. Hyperalgesia was decreased by the inhibition of TNFα within the dorsal root ganglia, and was found to be mediated by blocking p38 activation[415]. NGF is known to potentiate nociception and is up-regulated by IL-1β[416]. Macrophage-mediated Schwann cell denervation is mediated via MCP-1[417]. Utilizing knockout mice for the MCP-1 receptor CCR2, mechanical hyperalgesia does not develop after a partial nerve ligation[418 419]. Numerous studies have demonstrated allodynia in diabetes to be associated with Aβ and Aδ malfunction in sensory input[420 421].

Cannabinoid therapy not only alleviates the sensory complications of diabetic neuropathy, but can prevent its development. Numerous studies have identified phytocannabinoids, CB1, and CB2 as successful therapeutics and targets in treating neuropathic mechanical allodynia and thermal hyperalgesia[422 423 424 425 426 427 428 429 430 431 432 433 434 435]. Most of these studies have been conducted in animal models. Human and rodent neuropathies are identical in tactile features, as nerve injury induces homologous allodynic and hyperalgesic effects[436]. Furthermore, rodent models are considered more quantifiable in terms of hyperalgesia and effectiveness of treatment[437]. Nevertheless, clinical trial data has now been published that demonstrates a statistically significant reduction in neuropathic pain with the use of smoked marijuana[438].

In addition to neural synthesis of NGF, mast cells have been shown to secrete this factor as well[439]. NGF can also stimulate the secretion of numerous inflammatory proteins of mast cell origin, including itself, thus forming self up-

regulation[440 441 442 443 444]. Nociceptive signaling via NGF and its receptor trkA is attenuated via cannabinoid application[440 441]. Numerous primary afferent neurons express both CB1 and secrete NGF[445 446]. Both trkA and CB2 are located on the mast cell membrane[447], and the endocannabinoids PEA and AEA have been found to block trkA expression and prevent NGF mediated hyperalgesia[448 449].

We previously mentioned the association between allodynia and dysregulation of Aβ and Aδ inputs. CB1 expression displays an extremely high density within both these nociceptive fiber types[450 451 452]. Upon nerve injury within the periphery, numerous anatomical and immunohistochemical procedures identify an up-regulation of both CB1 and CB2[453 454 455]. Utilizing the CB1 antagonist SR141716A, mechanical allodynia and thermal hyperalgesia are increased in rodent models[423]. Cannabinoids inhibit neuropathic algesia via a similar mechanism to the most widely prescribed drug for the treatment of neuropathy, gabapentin[456 457]. Gabapentin, and other drugs commonly used to treat neuropathy, have all proven unsatisfactory in efficacy or are correlated to numerous harmful side effects[458 459 460 461]. Gabapentin reduces hyperalgesia via the inhibition of voltage-gated calcium channels of the L-, R-, P/Q-, I-, and N-types and subsequent intracellular reduction of Ca^{2+} [462 463]. Cannabinoids acting at the CB1 receptor have been demonstrated to inhibit Ca^{2+} currents in N-, P/Q-, and L- channel types[464 465 466 467].

In terms of preventing or blocking the mechanisms of neuropathy, cannabinoids act via multiple antioxidant and antiexcitotoxic pathways, thereby not only treating symptomology, but facilitating normal physiologic function in diabetic pathology. Both NMDA and β-amyloid neurotoxicity are attenuated by CBD[468]. It is a generally accepted trend that CB1 agonists are neuroprotective from ischemic and excitotoxic events[269 273 469 470 471]. The endogenous cannabinoid system is up-regulated during hypoxia and protects cells from oxidative damage[273]. These neuroprotective effects are also evident by the use of CB1 antagonists such as SR171416A, also known as Rimonabant. Under a model of NMDA induced neurotoxicity, the CB1 agonist Win55,212 reduced toxicity by 65%, an effect that was completely abolished by Rimonabant[472]. This same study found NMDA toxicity to be nNOS dependent, as activity at the NMDA receptor increased fluorescence of an NO tag by 160%. Win55,212 completely abolished NO production, an effect that was blocked by Rimonabant.

Cannabinoids prevent the formation of ROS and exert cellular protective effects via multiple mechanisms that are of benefit to diabetics. Neuroprotection is mediated via a PI3K/AKT dependent mechanism initiated by CB1 agonists, resulting in decreased p38 phosphorylation[473]. THC causes a decrease in p38 phosphorylation and a resulting inhibition in ROS formation and apoptosis[474]. Mitochondrial superoxide overproduction occurs in states of hyperglycemia[250]. The hexosamine pathway has been demonstrated to become activated by superoxides[475]. eNOS activity is inhibited by both superoxides and metabolites

of the hexosamine pathway[337]. The extremely high concentration of ROS in diabetics results from four major pathways, including the polyol, hexosamine, PKC, and advanced glycation end products (AGEs)[476]. NF-κβ activation has been correlated to all 4 pathways in addition to promoting endothelial leukocyte adhesion and up-regulation of Th1 inflammatory cytokines[237 476 477 478]. We previously discussed the cannabinoid mediated increase in eNOs activity, in addition to lowering the Th1 cytokine profile.

Ischemic-mediated production of ROS and subsequent cellular injury is found to be FeCl2 dependent[479 480]. Powerful oxides are formed from the release of Fe2+ via a Fenton reaction with H2O2. Using lactate dehydrogenase (LDH) as a marker of cortical neuronal apoptosis, FeCl2 within ischemic concentrations can induce as much as a 70% release of LDH from neurons[481]. When testing various cannabinoid receptor agonists under a model of FeCl2 induced neurotoxicity, apoptosis is reduced by as much as 50%[481]. The same study identified significant reductions in fluorescent detection of the oxidative product ethidium. Utilizing various antagonists and inhibitors, this research group identified the molecular mechanism of ROS inhibition by cannabinoids to be via inhibition of cAMP accumulation and subsequent PKA activation. Both cAMP and PKA have been implicated in the formation of ROS in neural and epithelial cells under states of excess activation[482]. Numerous cell lines are known to produce ROS from a PKA dependent mechanism including leptin activated epithelial cells, cardiomyocytes, and fibrosarcomas[483 484 485]. The CB1 receptor is well known for its ability to inhibit cAMP production and its stimulating effect on PKA phosphorylation[486]. Using the PKA activator dbcAMP or the CB1 antagonist Rimonabant, the neuroprotectant effect of CB1 agonists was completely abolished. PKA dependent ROS formation and oxidative damage from H202 and BSO were also found to be inhibited by CB1 agonists[481]. Further support for the CB1 receptor's role in ischemic damage lies in the discovery that it and the endogenous cannabinoid PEA are up-regulated after hypoxia and reduce the resulting inflammatory response[487 488].

We have discussed several neuroprotective effects of cannabinoid agonists that are receptor mediated, but potent antioxidant effects are exerted via non-receptor mediated mechanisms[489]. Overproduction of superoxides is known to occur in diabetic humans, in nearly every tissue type, including the retina, kidney, endothelium, nervous, and cardiovascular system[476 237]. Oxidative stress in diabetics can be evaluated via the degree of lipid peroxidation, of which, is typically elevated compared to non-diabetics[490]. In rodent models of neuropathy, prostaglandin E2 (PGE2) concentration is more than doubled, associated with this elevation is an increase in both lipid peroxidation and subsequent ROS[491].

The New York Academy of Sciences officially recognizes the potency of cannabinoids as antioxidants, quoting " In a head to head trail of abilities of various antioxidants to prevent glutamate toxicity, cannabidiol was superior to

both α-tocopherol an ascorbate in protective capacity"[272]. Specifically, this study found CBD to be 50% more potent an antioxidant than ascorbate. Utilizing numerous models, the Academy of Science determined that CBD can prevent H2O2 induced apoptosis by 75%. CBD has been found to be safe at such extremely high doses as 10mg/kg/day in human clinical trails with limited to no side effects[492]. This has a far safer therapeutic potential than even the over-the-counter antioxidant BHT, which even in small quantities has been linked to tumor formation[493 494].

Ischemic conditions result in excessive glutamatergic release creating neurotoxicity by overactivation of NMDA, kainate, or AMPA receptors by elevating intracellular Ca^{2+} to toxic levels[495]. Glutamatergic excitotoxicity is also ROS dependent[267 268]. These forms of toxicity have proven to be diminished with the administration of antioxidants both in vitro and in vivo[267 268]. Specifically, ischemic mediated ROS production can be alleviated by antioxidants such as α-tocopherol. Antioxidants can be of various structural forms as their distinctive feature is the ability to oxidize with ease. Ascorbate and tocopherols are among the best known. Besides the ability to oxidize, antioxidants have been demonstrated to inhibit iNOS and COX-2 transcription[496 497]. Cyclic voltammetry may not be an in vivo model, but its results can be extrapolated to such instances due to the quantitated measurement of the ability of the compound to donate or accept electrons. In yet another Ney York Academy of Sciences publication[272], cyclic voltammetry was used to assess the antioxidant potential of several vitamin antioxidants, BHT, THC, CBD, CBN, and HU-210. It was discovered that all the phytocannabinoids had equal or greater antioxidant potential than BHT. Under numerous cell models and protocols used to study antioxidant properties against ROS products of Fenton reactions, both THC and CBD were comparable or greater in efficacy to BHT. When comparing ascorbate, α-tocopherol, BHT, CBD, and THC in antioxidant effects against AMPA and kainite receptor excitotoxicity, CBD was found to have far superior properties than all others tested.

Cannabis may also exert antioxidant properties against the formation of AGEs. Marijuana from numerous landraces, cultivars, and hybrids have been found to contain significant concentrations of flavanoids, including quercitin and kaempferol[498]. As little as .5-10µg of quercitin or kaempferol can reduce hemoglobin glycosylation by as much as 52% and 15%, respectively[499].

8. Marijuana, Diabetes, and Depression:

A previous petition was submitted to include anxiety and depression on Amendment 20 and was denied due in part to an inability to link specific subtypes of depression to a specific mechanism of cannabinoid efficacy in treatment. Here we provide additional evidence to directly link at least one form of depression to benefits from marijuana.

New molecular evidence demonstrates a link between homologous modes of action in cannabinoid and Fluoxetine antidepressant efficacy. At the beginning of this paper and the discussion on neurological complications, we cite numerous in vivo, in vitro, and clinical studies demonstrating a high incidence of depression in diabetics. In animal models of type 1 diabetes, numerous complications are reported in the hippocampus, cerebral cortex, hypothalamus, and overall limbic system, including glutamatergic neurotoxicity, hippocampal cell death, decreased neurogenesis, and lowered synaptic plasticity[500] [501] [502] [503] [504]. Much of this pathology can be correlated to oxidative stress[505].

Human Type 1 diabetics have been demonstrated to display decreased hippocampal neurogenesis[506]. Interestingly, in a clinical trial treatment with Fluoxetine not only reduced depressive symptoms, but significantly brought glycemic values back into control[507]. As we have previously discussed the implications of cannabinoid neural stem/ progenitor cell neurogenesis within the hippocampus, reductions in cAMP, homology in mechanism to benzodiazepines and over a dozen antidepressants, we will not discuss the antidepressant effects of cannabinoids in detail here, but resubmit the depression petition as supporting evidence for this section. New evidence has been gathered however, that directly implicates efficacy of antidepressant drugs for various mood disorders and their mode of therapeutic efficacy being mediated by neurogenesis within the limbic structures[508] [509] [510] [511] [512] [513] [514] [515] [516].

9. Important Synergistic Interactions

It is noteworthy to mention that interactions between exogenous and endogenous cannabinoids can create a potent synergistic activity in numerous pathways for diabetic complications. Anandamide hydrolysis can be inhibited by CBD administration[317]. CBD also inhibits the metabolism of THC into 11-hydroxy THC, thus mediating a reduction in the psychoactive properties of the plant, as this THC metabolite displays significantly more potent psychological effects[306]. Thus all previously mentioned therapeutic effects of anandamide can be considered of benefit to the diabetic patient, as its influences are potentiated by phytocannabinoids.

10. Marijuana, Hyperglycemia, Hyperinsulinemia, β-Cell Regulation, and Insulin Signaling:

Numerous human clinical trials and animal models both find hyperglycemia to be an independent risk factor for the various microangiopathies correlated to diabetes[517] [518] [519] [520]. The pathogenic progression of T2D can be correlated to hyperinsulinemia, often the first detectable complication of the disease[521] [522] [523] [524] [525] [526] [527]. Abundant data has accumulated demonstrating the importance of insulin hypersecretion in the pathogenic progression of T2D[528]

[529] [530] [531] [532] [533] [534] [535]. Both insulin secretion and sensitivity are affected in the hyperinsulinemic state[536] [537]. Hyperinsulinemia precedes the onset of T2D[538] [539] [540].

Hyperglycemia is also responsible for insulin resistance, in addition to its contributions to all the various complications associated with both forms of diabetes mellitus. As a coping mechanism, T-lymphocytes develop insulin receptors under hyperglycemic conditions, with concomitant lipid peroxidation and resulting ROS production[541] [542] [543] [544] [545] [546]. Diabetic patients in ketoacidosis display significantly higher levels of TNFα, IL-1β, IL-1βR, IL-8, and CRP[547]. The AKT signaling pathway activates eNOS[237] [427]. Hyperglycemia inhibits eNOS activity via hexosamine metabolites direct O-linked glycosylated modifications to the AKT protein[337].

Insulin secretion occurs in a pulsatile manner within the β-cell, with the opening of VGCCs allowing an intracellular accumulation of Ca^{2+} [548] [549] [550]. VGCC closure and subsequent inhibition of intracellular Ca^{2+} levels is a well documented feature associated with agonist activity at both CB receptors[464] [465] [551] [552] [553].

As we shall demonstrate, marijuana may reduce the harmful effects of hyperinsulinemia, increase glucose metabolism, and enhance insulin signaling. Both cannabinoid receptors have been identified within islet cells particularly CB1 being predominant on α-cells while CB2 is localized to both α- and β-cells[554]. CB2 agonists such as anandamide, 2-AG, methanandamide, and JWH have been demonstrated to reduce glucose-evoked insulin secretion by as much as 30%[554]. Effects were abolished by administration of the CB2 antagonist AM630.

At first consideration one might view this effect as harmful, however, the additional metabolic activities of cannabinoids in conjunction with decreased insulin secretion gives an overall benefit by enhancing glucose uptake without requiring additional insulin. Glucose uptake increases by as much as 160% in 3T3-L1 adipocytes pretreated with AEA (anandamide)[555]. The CB1 selective agonist ACEA enhances glucose uptake in human endothelial cells[556]. In vivo stimulation of glucose uptake in numerous tissues, including skeletal muscle, adipose, and endothelial, is NOS dependent[557]. Anandamide, and other CB1 agonists previously discussed in the NOS section can increase NOS in these tissues[558].

Arachidonic acid (AA) enhances both basal and insulin stimulated glucose catabolism while COX-2 synthesized products of AA inhibit glucose metabolism[559] [560] [561]. We had previously discussed the cannabinoid mediated benefits of COX-2 inhibition.

Activation of the PI3K and AKT/PKB signaling pathways is a well documented phenomenon associated with CB receptor signaling[562] [563] [564].

Specifically, Rimonabant has been demonstrated to inhibit the IR stimulated activation of PI3K via ERK[564]. This demonstrates unequivocally at the exact phosphorylation sites and signaling proteins that cannabinoids have a homologous signaling pathway of medical benefit to the IR signaling pathway. Signaling pathways of both the CB1 and insulin receptors converge at ERK phosphorylation[565]. Activation of either CB1 or the IR causes phosphorylation of $PI3K_{1b}$, an effect which is blocked from both signaling pathways by the $PI3K_{1b}$ inhibitor Wortmannin[564]. Additionally, numerous studies show support for a CB1 mediated phosphorylation of ERK[566] [567] [568]. As the PI3K/AKT pathway is responsible for GLUT-4 translocation, in addition to numerous other metabolically beneficial actions resulting from the anti-apoptotic signals of ERK, we find that CB activation can enhance the effects of insulin signaling while CB2 activation results in reduced insulin secretion.

11. Personal Testimony/ Anecdotal Evidence:

As a 23 year diabetic with severe gastroparesis and sensory neuropathy, I know all to well the pain, nausea, and debilitations my disease has imposed upon me. I began my college career in 2000, only to withdraw a year later due to an inability to drive, a constant need for a bathroom, and general feelings of discomfort (tactile sensitivity). As a once recreational user of cannabis, I used the drug on occasion. On one such event, my recreational use coincided with a day of extreme pain and vomiting. In less than 5 minutes of inhalation, my nausea went away completely, and my pain became more of a minor pressure than burning sensation. Since then I have used medical marijuana under the guidance of my doctor. With repeated, consistent use at regularly scheduled times I also noticed the effects that marijuana had on my blood-glucose levels. I found that smoking marijuana lowered my glucose levels, so much that I began lowering my insulin dosage for the first time in years.

Diabetic ketoacidosis is a truly horrific feeling throughout the body. I often describe it as liquid mosquito bites pumping throughout my veins. Even with consistent Glycohemoglobin A1C levels between 6-7, hyperglycemia affects me 1-2x weekly. Depending on the severity, I may be bedridden for up to 2 days with flu-like symptoms. Smoking marijuana after ketoacidosis dramatically reduces my symptoms, not completely abolishing, but reducing enough such that I may be productive again in a few hours.

There are also times I must eat food due to hypoglycemia, but vomit the food up due to gastroparesis. Cannabis allows me to both hold food down and it stimulates hunger such that I can eat when necessary.

I have a fiancée who loves me unconditionally. I am often frustrated and ashamed because of a frequency with premature ejaculation and impotence. At first this created problems in our relationship, mostly with my own anger and depression with my personal inadequacies. On nights when I must medicate for

nausea or pain related reasons, I sometimes find that marijuana helps me to maintain an erection longer.

I do not feel that marijuana is a perfect therapy for diabetes. One problem I find is that I must be disciplined not to eat carbohydrates after medicating: a phenomenon known all to well as the munchies. This emphasizes the importance of marijuana as a medicine and not a recreational drug. A recreational user would act on "the munchies", whereas a medical patient would be receiving A1Cs and complete bloodwork from their endocrinologist to identify how well they are managing the use of their medicine and diet.

12. Summary:

Diabetes is a debilitating condition due to the numerous pathologies and diseases it predisposes the patient to as a result of its progression. Diabetes is also considerd the 305th largest cause of death in the United States. Death can be considered the ultimate form of debilitation, thus if cannabis can prevent those complications which contribute to the death of diabetics, the treatment should be considered. The American Diabetes Association, World Health Organization, and numerous studies find an overwhelming incidence of neurologic and metabolic disorders arising in this population. Virtually all diabetics face the fact that neuropathy, blindness, pain, nausea, improper digestion, depression, gastroparesis, sexual dysfunction, and cardiovascular diseases are all possible and highly probable developments in diabetes mellitus. Furthermore, the ADA believes these pathologies to occur similiarly in both Types 1 and 2 of diabetes mellitus, with a quicker progression more evident in Type 1 than 2.

Diabetes is clearly a debilitating and diagnosable disease. A simple glucose tolerance test can determine if a patient is diabetic; a simple ELISA test for CRP or insulin can differentiate between the two subtypes. Although there has been a recent discussion on updating the cut-off values of blood glucose levels in determining a diabetic from a non-diabetic, these values do not vary drastically from the still upheld values determined in the 1980s.

We have discussed numerous metabolic, inflammatory, neurologic, retinal, and free radical pathways resulting in numerous pathologies to various tissues in the diabetic state. Focusing on the molecules as groups in lieu of their mechanisms and tissues, we find:

1. Inflammatory proteins are produced from an up-regulation of Th1. They can also come from adipose tissue, macrophages, neurons, and COX enzymes. Cannabinoids balance the Th1/Th2 balance either towards equilibrium or towards a Th2/Th1 ratio. Numerous cannabinoids were demonstrated to inhibit TNFα, IL-1β, IL-6, as well as benefits against JAK-STAT, JNK, and SOCS protein signaling. We identified how these proteins inhibit numerous

activities associated with the insulin receptor and included specific molecular mechanisms of insulin resistance associated with these actions.

2. We also identified how FFAs share similar signaling pathways to these inflammatory proteins, in addition to direct actions of FFAs increasing transcription and/or translation of inflammatory cytokines, thus proving a cyclic upregulation to the pathology. With numerous citations, we demonstrate that cannabinoids work similar to TZDs in reducing insulin resistance but concomitantly increasing adiposity. Marijuana increases weight similar to TZDs; reducing blood glucose concentration via adipocyte lipogenesis.
3. Hyperglycemia favors AA metabolism via COX-2 in numerous tissues, particularly the retina. These inflammatory mediators are associated with increase in free radicals, VEGF, and numerous retinal pathologies. We identified how marijuana products, both of cannabinoid and non-cannabinoid structure can inhibit COX-2 activity, reduce the specific free radicals associated with diabetic retinopathy, and inhibit angiogenesis via down-regulation of VEGF.
4. Hyperglycemia also causes systemic up-regulation of pro-inflammatory cytokines and increases the concentration of numerous free radicals of multiple pathways. Again, we identify how marijuana reduces COX, PKA, excitotoxic, NMDA, hexosamine, NF-κβ, interleukin, TNFα originated mechanisms of free radical production. These free radicals are associated with many of the neurodegenerative effects viewed in diabetics. Additionally, by preventing these free radicals, cannabis helps protect the diabetic from the 3 common microangiopathies: neuropathy, retinopathy, and nephropathy.
5. Hyperglycemia causes numerous cytokines and immune-derived adhesion molecules to become up-regulated, causing the dramatically high incidence of atherosclerosis in diabetics. Here we identified that marijuana inhibits MCP-1 and several adhesion molecules. CB1 and CB2 receptors have been found to become up-regulated on atherosclerotic plaques, agonists are found to decrease progression of plaque formation.
6. Diabetics are far more likely to die from hypertension related complications than the average individual. In line with preventing atherosclerosis via inducing hypertension, the synergistic activities between endo- and exogenous cannabinoids results in increased cardiac output, vasodilation, and increased heart rate with a drop in BP. Every one of these actions are beneficial to diabetic cardiovascular physiology, when combined as a whole.
7. We identified the unique activites of cannabinoids on NOS activities. Interestingly, marijuana constituents have the ability to activate eNOS, increase iNOS in the skeletal muscle and endothelial tissue, and decrease iNOS and nNOS in the nervous

and immune systems. Thus cannabinoids help prevent nNOS directed neurodegeneration and peripheral iNOS mediated increases in BP, while increasing glucose metabolism in the skeletal muscle and endothelial tissues.

8. We identified a depletion of naturally occurring antioxidants in diabetics in addition to free radical based damages of every major organ system in the human body. Cyclic voltammetry identifies both THC and CBD as at least 20x more potent of antioxidants than α-tocopherol or ascorbate.
9. We identified specific interrelationships between signaling pathways of the CB and insulin receptors. Cannabinoids can decrease hyperinsulinemia while simultaneously increasing glucose metabolism in numerous tissues.

13. Conclusion:

In conclusion, we find overwhelming evidence to support that marijuana ***may*** have a beneficial effect in the treatment of diabetes mellitus of both Type 1 and 2. Due to its schedule I classification clinical trials with marijuana are nearly impossible to perform legally, and the request for human clinical trials by Mr Cologne of the department of health is unacceptable and impossible to fulfill. Here, we utilize numerous studies of in vitro and in vivo to demonstrate a vast multitude of strongly supported mechanisms of therapeutic benefit to the diabetic based on a strong foundation of peer-reviewed support from the literature.

When deciding proper vocabulary to utilize in a legal statute, nonetheless a constitutional amendment, choosing the word "***may***" implies a significant and substantial room for discussion. If Amendment 20 used the word "***must***", this would imply unequivocal, double blind clinical trial, peer reviewed work on large sample populations. "May" is a far broader definition than "must", and as the Colorado Constitutional Amendment 20 uses the word "***may***", review of any petition being submitted under the context of Amendment 20 "***must"*** be reviewed in this broader context, less infringement of Constitutional Rights be the discussion of this petitions review by a judiciary committee.

We thank the Colorado Department of Public Health and Environment for their time and efforts in review of this petition.

[1] Alberti, K.G. and Zimmet, P.Z. 1998. "Definition, diagnosis, and classification of diabetes mellitus and its complications part 1: Diagnosis and classification of diabetes mellitus provisional report of a WHO consultation". Diabetic Medicine 15: 539-553.
[2] World Health Organization. 1985. "Diabetes mellitus: Report of a WHO study group". Technical Report Series 727.
[3] Hother-Nielson, O., et al. 1988. "Classification of newly diagnosed diabetic patients as insulin-requiring or non-insulin requiring based on clinical and biochemical variables". Diabetes Care 11: 531-537.
[4] DeFronzo, R.A., et al. 1997. "Pathogenesis of IDDM". International textbook of Diabetes Mellitus, 2nd Ed: 635-712.
[5] Lillioja, S., et al. 1993. "Insulin resistance and insulin secretory dysfunction as precursors of non-insulin dependent diabetes. Prospective study of Pima Indians". New England Journal of Medicine 329: 1988-1992.
[6] Campbell, P.J., and Carlson, M.G. Impact of obesity on insulin action in NIDDM". Diabetes 42: 405-410.
[7] Kissebah, A.H., et al. 1982. "Relationship of body fat distribution to metabolic complications of obesity". Journal of Clinical Endocrinology and Metabolism 54: 254-260.
[8] Valle, T. et al. 1997. 'Epidemiology of NIDDM in Europids". International Textbook of Diabetes Mellitus, 2nd Edition: 125-142.
[9] Knowler, W.C., et al. 1993. "Determinants of diabetes mellitus in the Pima Indians." Diabetes Care 16: 216-227.
[10] Neely, K.A. and Gardner, T.W. 1998. Ocular neovascularization: clarifying complex interactions". American Journal of Pathology 153: 665-670.
[11] Ferris, F.L., et al. 1999. "Treatment of diabetic retinopathy". Ne England Journal of Medicine 341: 667-678.
[12] Vinik, A.I., et al. 1992. "Diabetic neuropathies". Diabetes Care 15: 1926-1975.
[13] Siddique, R. Nguyen, M., and Farup, C. 1998. " Cost of hospitalization for diabetic patients with vomiting: evidence from a national survey". Gastroenterology 114: A41.
[14] Smith, D.S., and Ferris, C.D. 2003. "Current concepts in diabetic gastroparesis". Drugs 63: 1339-1358.
[15] Grewal, J., Bril, V., et al. 2006. Objective evidence for the reversibility of nerve injury in diabetic neuropathic cachexia. Diabetes Care 29(2): 473-474.
[16] Denton, RM, Brownstein, RW, and Belsham, GI. 1981. "A partial view of the mechanism of insulin action." Diabetologia 21: 347-362.
[17] Paz, K. et al. 1997. "A molecular basis for insulin resistance: Elevated serine/threonine phosphorylation of IRS-1 and IRS-2 inhibits their binding to the juxtamembrane region of the insulin receptor and impairs their ability to undergo insulin-induced tyrosine phosphorylation." Journal of Biological Chemistry 272: 29911-29918.
[18] Rosen, OM, et al. 1982. "Phosphorylation activates the insulin receptor tyrosine protein kinase." Journal of Biological Chemistry 80: 3237-3240.
[19] Lam, K, et al. 1994. "The phosphatidylinositol-3 kinase serine kinase phosphorylates IRS-1. Stimulation by insulin and inhibition by Wortmannin." Journal of Biological Chemistry 269: 20648-20652.

[20] Rondinone, CM, et al. 1997. "Insulin receptor substrate (IRS) 1 is reduced and IRS-2 is the main docking protein for phosphoinositol-3 kinase in adipocytes from subjects with non-insulin dependent diabetes mellitus." Proceedings of the National Academy of Sciences USA 94: 4171-4175.
[21] Kasuga, M. et al. 1982. "Insulin stimulation of phosphorylation of the beta subunit of the insulin receptor." Journal of Biological Chemistry 257: 9891-9899.
[22] Zick, Y, et al. 1983. "Insulin stimulates phosphorylation of serine residues in soluble insulin receptors." Biochemistry Biophysics Resources Communications 116: 1129-1135.
[23] Tsuruzoe, R, et al. 2001. "Insulin receptor substrate 3 (IRS-3) and IRS-4 impair IRS-1 and IRS-2 mediated signaling." Molecular Cellular Biology 21: 26-38.
[24] Isseman, I, et al. 1993. "The retinoic X receptor enhances the function of peroxisome proliferators activated receptor." Biochimie 75: 251-256.
[25] Keller, H, et al. 1993. "Fatty acids and retinoids control lipid metabolism through activation of peroxisome proliferators activated receptor gamma and retinoic X receptor heterodimerization." Proceedings National Academy of Sciences USA 90: 2160-2164.
[26] Rangwalla, SM and Lazar, MA. 2004. "Peroxisome proliferators activated receptor gamma in diabetes and metabolism." Trends in Pharmacological Science 25: 331-336.
[27] Yamamoto, H., et al. 1981. "Streptozotocin and alloxan induce DNA strand breaks and poly(ADP-ribose) synthetase in pancreatic islets". Nature 294: 284-286.
[28] Xinguang, Li, et al. 2001. "Examination of the immunosuppressant effects of tetrahydrocannabinol in streptozotocin-induced autoimmune diabetes". International Immunopharmacology 1: 699-712.
[29] Maclaren NK, Alkinson MA. 1997. "Insulin-dependent diabetes mellitus: the hypothesis of molecular mimicry between islet cell antigens and microorganisms". Molecular Medicine Today 3: 76-83.
[30] Rabinovitch A. 1994. Immunoregulatory and cytokine imbalances in the pathogenesis of IDDM. Diabetes 43:613–21.
[31] McGarry J. 2002**.** Dysregulation of fatty acid metabolism in theetiology of type 2 diabetes. *Diabetes* 51: 7–18.
[32] Reaven, G. M., et al. 1988. Measurement of plasma glucose, free fatty acid, lactate, and insulin for 24h in patients with NIDDM. Diabetes 37: 1020– 1024.
[33] Boden, G., et al. 1991. Effects of fat on insulin-stimulated carbohydrate metabolism in normal men. Journal of Clinical Investigation 88: 960– 966.
[34] Hawkins, M., et al. 2003. Contribution of elevated free fatty acid levels to the lack of glucose effectiveness in type 2 diabetes. Diabetes 52: 2748– 2758.
[35] Bays, H., Mandarino, L., and DeFronzo, R. A. 2004. *J. Clinical Endocrinolology Metababolism* 89: 463–478
[36] Roden, M. 2001. Non-invasive studies of glycogen metabolism in human skeletal muscle using nuclear magnetic resonance spectroscopy. Current Opinion Clinical Nutrition Metabolism Care 4, 261– 266.
[37] Boden G, Chen X, Ruiz J, White JV, and Rossetti L**.** 1994. Mechanisms of fatty acid-induced inhibition of glucose uptake. *Journal of Clinical Investigation* 93: 2438–2446.

[38] Chalkley SM, Hettiarachchi M, Chisholm DJ, and Kraegen EW. 1998. Five-hour fatty acid elevation increases muscle lipids and impairs glycogen synthesis in the rat. *Metabolism* 47: 1121–1126.
[39] Kelley DE, Mokan M, Simoneau JA, and Mandarino LJ. 1993. Interaction between glucose and free fatty acid metabolism in human skeletal muscle. *Journal of Clinical Investigation* 92: 91–98.
[40] Vaag, A., et al. 1991. Effect of the antilipolytic nicotinic acid analogue acipimox on whole-body and skeletal muscle glucose metabolism in patients with noninsulin-dependent diabetes mellitus. Journal of Clinical Investigation 88: 1282– 1290.
[41] Kraegen, E. W., Cooney, G. J., Ye, J. M., Thompson, A. L., and Furler, S. M. 2001. *Experimental Clinical Endocrinology Diabetes:* 109, Suppl. 2, 189–201.
[42] Boden, G. 1997. Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. *Diabetes* 46: 3–10.
[43] Riccardi, G., and Rivellese, A. A. 2000. Dietary treatment of the metabolic syndrome. *British Journal of Nutrition* 83, Suppl. 1, 143–148
[44] Yu, C., et al. 2002. Mechanism by which fatty acids inhibit insulin activation of insulin receptor substrate-1 (IRS-1)-associated phosphatidylinositol-3 kinase activity in muscle. *Journal of Biological Chemistry* 277: 50230–5023.
[45] Hirosumi, J., et al. 2002. A central role for JNK in obesity and insulin resistance. *Nature* 420: 333–336.
[46] Paz, K. et al. 1997. "A molecular basis for insulin resistance: Elevated serine/threonine phosphorylation of IRS-1 and IRS-2 inhibits their binding to the juxtamembrane region of the insulin receptor and impairs their ability to undergo insulin-induced tyrosine phosphorylation." Journal of Biological Chemistry 272: 29911-29918.
[46] Rondinone, CM, et al. 1997. "Insulin receptor substrate (IRS) 1 is reduced and IRS-2 is the main docking protein for phosphoinositol-3 kinase in adipocytes from subjects with non-insulin dependent diabetes mellitus." Proceedings of the National Academy of Sciences USA 94: 4171-4175.
[47] Griffin, M. E., et al. 1999. *Diabetes* 48: 1270–1274.
[48] Gao, Z., et al. 2004. Inhibition of insulin sensitivity by free fatty acids requires activation of multiple serine kinases in 3T3-L1 adipocytes. Molecular Endocrinology 18, 2024–2034.
[49] Moeschel, K., et al. 2004. Protein kinase C-zeta-induced phosphorylation of Ser318 in insulin receptor substrate-1 (IRS-1) attenuates the interaction with the insulin receptor and the tyrosine phosphorylation of IRS-1. Journal of Biological Chemistry 279: 25157–25163.
[50] Nguyen, M.T., et al. 2005. JNK and tumor necrosis factor-alpha mediate free fatty acid induced insulin resistance in 3T3-L1 adipocytes. Journal of Biological Chemistry 280(42): 35361-35371.
[51] Thompson A, Lim-Fraser MYC, Kraegen EW, and Cooney GJ. 2000. Effects of individual fatty acids on glucose uptake and glycogen synthesis in soleus muscle in vitro. *American Journal of Physiology Endocrinology Metabolism* 279: E577–E584.
[52] Kim JB, Shulman GI, and Kahn BB. Fatty acid infusion selectively impairs insulin action on Akt1 and PKC/ but not on glycogen synthase kinase-3. *J Biol Chem.* In press.

[53] Dresner A, Laurent D, et al. 1999. Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol3-kinase activity. *Journal of Clinical Invesigationt* 103: 253–259.
[54] Subtil, A., Lampson, M. A., Keller, S. R., and McGraw, T. E. 2000. *Journal of Biological Chemistry* 275: 4787–4795.
[55] Ruan, H., and Lodish, H. F. 2003. *Cytokine Growth Factor Review* 14: 447–455.
[56] Peraldi, P., and Spiegelman, B. 1998. *Mol. Cell Biochemistry* 182: 169–175.
[57] Xu, H., Uysal, K. T., Becherer, J. D., Arner, P., and Hotamisligil, G. S. 2002). *Diabetes* 51: 1876–1883.
[58] Hotamisligil, G. S., et al. 1995. Increased adipose tissue expression of tumor necrosis factor-alpha
in human obesity and insulin resistance. Journal of Clinical Investigation 95: 2409–2415.
[59] Hotamisligil, G. S., Shargill, N. S., & Spiegelman, B. M. 1993. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 259: 87– 91.
[60] Borst, S. E. 2004. The role of TNF-alpha in insulin resistance. Endocrine 23: 177–182.
[61] Kyriakis JM, Avruch J. 2001. Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. Physiol Rev 81:807–69.
[62] Aguirre V, Uchida T, Yenush L, Davis R, White MF. 2000. The c-Jun NH(2)-terminal kinase promotes insulin resistance during association withinsulin receptor substrate-1 and phosphorylation of Ser(307). Journal of BiologicalChemistry 275:9047–54.
[63] Hirosumi J, Tuncman G, Chang L, Gorgun CZ, Uysal KT, Maeda K, et al. 2002. A central role for JNK in obesity and insulin resistance. Nature 420:333–6.
[64] Nakatani Y, Kaneto H, Kawamori D, Yoshiuchi K, Hatazaki M, Matsuoka TA, et al. 2005. Involvement of endoplasmic reticulum stress in insulin resistance and diabetes. Journal of Biological Chemistry 280:847–51.
[65] Zagariya, A., Mungre, S., et al. 1998. *Mol. Cell. Biol.* 18: 2815–2824.
[66] Ryden, M., Dicker, A., et al. 2002. Mapping of early signaling events in tumor necrosis factor-alpha-mediated lipolysis in human fat cells. Journal of Biological Chemistry 277: 1085– 1091.
[67] Green, A., Dobias, S. B., Walters, D. J., & Brasier, A. R. 1994. Tumor necrosis factor increases the rate of lipolysis in primary cultures of adipocytes without altering levels of hormone-sensitive lipase. Endocrinology 134: 2581–2588.
[68] Ryden, M., Arvidsson, E., et al. 2004. Targets for TNF-alpha-induced lipolysis in human adipocytes.
Biochemistry Biophysics Resources Communications 318, 168– 175.
[69] Feingold, K. R., Doerrler, W., Dinarello, C. A., Fiers, W., and Grunfeld, C. 1992. *Endocrinology* **130:** 10–16.
[70] Rosenstock, M., Greenberg, A. S., and Rudich, A. 2001. *Diabetologia* **44:** 55–62.
[71] Gasic, S., Tian, B., and Green, A. 1999. *Journal of Biological Chemistry* **274,** 6770–677.
[72] Hauner, H., Petruschke, T., Russ, M., Rohrig, K., and Eckel, J. 1995. *Diabetologia* **38:** 764–771.

[73] Hotamisligil GS, Spiegelman BM. 1994. Tumor necrosis factor alpha: a key component of the obesity-diabetes link. *Diabetes* 43:1271–1278.
[74] Miles PD, Romeo OM, Higo K, Cohen A, Rafaat K, Olefsky JM. 1997. TNF-alpha induced
insulin resistance in vivo and its prevention by troglitazone. *Diabetes* 46:1678–1683.
[75] Hotamisligil GS, Shargill NS, Spiegelman BM. 1993. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* 259:87–91.
[76] Hofmann C, Lorenz K, Braithwaite SS, Colca JR, Palazuk BJ, Hotamisligil
GS, Spiegelman BM. 1994. Altered gene expression for tumor necrosis facto ralpha and its receptors during drug and dietary modulation of insulin resistance. *Endocrinology* 134:264–270.
[77] Hamann A, Benecke H, Le Marchand-Brustel Y, Susulic VS, Lowell BB,
Flier JS. 1995. Characterization of insulin resistance and NIDDM in transgenic mice with reduced brown fat. *Diabetes* 44:1266–1273.
[78] Kern PA, Saghizadeh M, Ong JM, Bosch RJ, Deem R, Simsolo RB. 1995. Theexpression of tumor necrosis factor in human adipose tissue: regulation byobesity, weight loss, and relationship to lipoprotein lipase. *Journal of Clinical Investigation* 95:2111–2119.
[79] Lang, C. H., Dobrescu, C., & Bagby, G. J. 1992. Tumor necrosis factor impairs insulin action on peripheral glucose disposal and hepatic glucose output. Endocrinology 130: 43– 52.
[80] Ruan, H., and Lodish, H. F. 2003. *Cytokine Growth Factor Rev.* 14: 447–455.
[81] Kappes, A., and Loffler, G. (2000) *Hormone Metabolism Reearch.* 32: 548–554
[82] Krebs, D. L., and Hilton, D. J. 2003. *Science. STKE* , PE6.
[83] Emanuelli, B., Peraldi, P., et al. 2001. *Journal of Biological Chemistry* 276: 47944–47949.
[84] Ueki, K., Kondo, T., and Kahn, C. R. 2004. *Mol. Cell. Biol.* 24, 5434–5446.
[85] Rui, L., Yuan, M., Frantz, D., Shoelson, S., and White, M. F. 2002. *Journal of Biological Chemistry* 277: 42394–42398.
[86] Ueki K, Kadowaki T, Kahn CR. Role of suppressors of cytokine signaling SOCS-1 and SOCS-3 in hepatic steatosis and the metabolicsyndrome. 2005. Hepatology Res .
[87] Stephens, J. M., Lee, J., & Pilch, P. F. 1997. Tumor necrosis factor-alpha induced insulin resistance in 3T3-L1 adipocytes is accompanied by a loss of insulin receptor substrate-1 and GLUT4 expression without a loss of insulin receptor-mediated signal transduction. Journal of Biological Chemistry 272: 971– 976.
[88] Hotamisligil, G. S., Murray, D. L., Choy, L. N., & Spiegelman, B. M. 1994b. Tumor necrosis factor alpha inhibits signaling from the insulin receptor. Proceedings of the National Academies Science U S A 91: 4854– 4858.
[89] Valverde, A. M., Teruel, T., Navarro, P., Benito, M., & Lorenzo, M. 1998. Tumor necrosis factor-alpha causes insulin receptor substrate-2-mediated insulin resistance and inhibits insulin-induced adipogenesis in fetal brown adipocytes. Endocrinology 139: 1229– 1238.

[90] Hotamisligil, G. S., Budavari, A., Murray, D., & Spiegelman, B. M. 1994a. Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes. Central role of tumor necrosis factor-alpha. Journal of Clinical Investigation 94: 1543– 154.
[91] Ragolia, L., & Begum, N. 1998. Protein phosphatase-1 and insulin action. Mol Cell Biochem 182, 49– 58.
[92] Liu LS, Spelleken M, Rohrig K, Hauner H, Eckel J. 1998. Tumor necrosis factor-alpha acutely inhibits insulin signaling in human adipocytes: implication of the p80 tumor necrosis factor receptor. *Diabetes* 47:515–522.
[93] Feinstein R, Kanety H, Papa MZ, Lunenfeld B, Karasik A. 1993. Tumor necrosis factor-alpha suppresses insulin-induced tyrosine phosphorylation of insulin receptor and its substrates. *J Biol Chem* 268:26055–26058.
[94] Ozes ON, Akca H, Mayo LD, Gustin JA, Maehama T, Dixon JE, Donner DB. 2001. A phosphatidylinositol 3-kinase/Akt/mTOR pathway mediates and PTEN antagonizes tumor necrosis factor inhibition of insulin signaling through insulin receptor substrate-1. *Proc Natl Acad Sci U S A* 98:4640–4645.
[95] Kanety, H., Feinstein, R., Papa, M. Z., Hemi, R., & Karasik, A. 1995. Tumor necrosis factor alpha-induced phosphorylation of insulin receptor substrate-1 (IRS-1). Possible mechanism for suppression of
insulin-stimulated tyrosine phosphorylation of IRS-1. Journal of Biological Chemistry 270: 23780–23784.
[96] Hotamisligil, G. S., Peraldi, P., et al. 1996. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-a-and obesity-induced insulin resistance. Science 271: 665–668.
[97] Pederson, T. M., Kramer, D. L., & Rondinone, C. M. 2001. Serine/threonine phosphorylation of IRS-1 triggers its degradation: possible regulation by tyrosine phosphorylation. Diabetes 50: 24–31.
[98] Calderhead, D.M., et al. 1990. Insulin regulation of the two glucose transporters in 3T3-Ll adipocytes. J Biol Chem 265:13800-13808
[99] Yang J, Clark AE, Kozka IJ, Cushman SW, Holman GD. 1992. Development of an intracellular pool of glucose transporters in 3T3-Ll cells. J Biol Chem 267: 10393-10399.
[100] Torti FM, Torti SV, et al. 1989. Modulation of adipocyte differentiation by tumor necrosis factor and transforming growth factor beta. J Cell Biol 108:1105-1113.
[101] Petruschke TH, Hauner H. 1993. Tumor necrosis factor-o prevents the differentiation of human adipocyte precursor cells and causes delipidation of newly developed fat cells. J Clin Endocrinol Metab
76~742-747.
[102] Weiner, F.R., et al. 1989. Regulation of collagen gene expression in 3T3-Ll cells. Effects of adipocyte differentiation and tumor necrosis factor 0. Biochemistry 284094-4099.

[103] Ruan, H. et al. 2002. Tumor necrosis factor alpha suppresses adipocyte specific genes and activates expression of preadipocyte genes in 3T3-L1 adipocytes. Diabetes 51: 1319-1336.
[104] Berg AH, Combs TP, Du X, Brownlee M, Scherer PE. 2001. The adipocyte secreted protein Acrp30 enhances hepatic insulin action. *Nat Med* 7: 947–953.

[105] Fruebis J, Tsao TS, Javorschi S, Ebbets-Reed D, Erickson MR, Yen FT, Bihain BE, Lodish HF. 2001. Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. *Proc Natl Acad Sci U S A* 98:2005–2010.
[106] Stephens JM, Lee J, Pilch PF. 1997. Tumor necrosis factor-alpha-induced insulin resistance in 3T3–L1 adipocytes is accompanied by a loss of insulin receptorsubstrate-1 and GLUT4 expression without a loss of insulin receptor mediated signal transduction. *J Biol Chem* 272:971–976.
[107] Hotamisligil GS, Peraldi P, et al. 1996. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. *Science* 271:665–668.
[108] Hamann A, Benecke H, Le Marchand-Brustel Y, Susulic VS, Lowell BB, Flier JS. 1995. Characterization of insulin resistance and NIDDM in transgenic mice with reduced brown fat. *Diabetes* 44:1266–1273.
[109] DeFronzo RA, Gunnarsson R, et al. 1985. Effects of insulin on peripheral and splanchnic glucose metabolism in noninsulin-dependent (type II) diabetesmellitus. *J Clin Invest* 76: 149–155.
[110] Steinberg, G.R., Macaulay, S.L., Febbraio, M.A., and Kemp, B.E. 2006. AMP-Kinase the fat controller of the energy railroad. Can. J. Phys. Pharm. 84: 655–665.
[111] Ruderman, N.B., Saha, A.K., Vavvas, D., and Witters, L.A. 1999 . Malonyl-CoA, fuel sensing, and insulin resistance. American Journal of Physiology 276: E1–E18.
[112] Merrill GF, Kurth EJ, Hardie DG, and Winder WW. 1997. AICA riboside increases AMP-activated protein kinase, fatty acid oxidation, and glucose uptake in rat muscle. *Am J Physiol Endocrinol Metab* 273: E1107–E1112.
[113] Zong, H., Ren, J.M., et al. 2002. AMP kinase is required for mitochondrial biogenesis in skeletal muscle in response to chronic energy deprivation. Proc. Natl. Acad. Sci. USA 99, 15983–15987.
[114] Bergeron R, Russell RR, Young LH, Ren JM, Marcucci M, Lee A, and Shulman GI. 1999. Effect of AMPK activation on muscle glucose metabolism in conscious rats. *Am J Physiol Endocrinol Metab* 276: E938–E944, 1999.
[115] Hayashi T, Hirshman MF, Kurth EJ, Winder WW, and Goodyear LJ. 1998. Evidence for 5_AMP-activated protein-kinase mediation of the effect of muscle contraction on glucose transport. *Diabetes* 47: 1369–1373.
[116] Kurth-Kraczek EJ, Hirshman MF, Goodyear LJ, and Winder WW. 1999. 5_-AMP-activated protein kinase activation causes GLUT4 translocation in skeletal muscle. *Diabetes* 48: 1667–1671.
[117] Kemp, B.E., Stapleton, D., Campbell, D.J., et al. 2003. AMP activated protein kinase, super metabolic regulator. Biochem. Soc. Trans. 31: 162–168.
[118] Adams, J., Chen, Z.-P., et al. 2004. Intrasteric control of AMPK via the {gamma}1 subunit AMP allosteric regulatory site. Protein Sci. 13, 155–165.
[119] Cheung, P.C., Salt, I.P., et al. 2000. Characterization of AMP-activated protein kinase gamma-subunit isoforms and their role in AMP binding. Biochem. J. 346, 659–669.
[120] Davies, S.P., Helps, N.R., Cohen, P.T., and Hardie, D.G. 1995. 50-AMP inhibits dephosphorylation, as well as promoting phosphorylation, of the AMP-activated protein

kinase. Studies using bacterially expressed human protein phosphatase-2C alpha and native bovine protein phosphatase- 2AC. FEBS Lett. 377: 421–425.
[121] Fujii N, Hayashi T, Hirshman MF, et al. **2000.** Exercise induces isoform specific increase in 5 _ AMP- activated protein kinase activity in human skeletal muscle. *Biochem Biophys Res Commun* 273:1150–1155.
[122] Winder, WW and Hardie DG**.** 1996. Inactivation of acetyl-CoA carboxylase and activation of AMP-activated protein kinase in muscle during exercise. *Am J Physiol Endocrinol Metab* 270: E299–E304.
[123] Winder WW and Hardie DG**.** 1999. AMP-activated protein kinase, a metabolic master switch: possible roles in type 2 diabetes. *Am J Physiol Endocrinol Metab* 277: E1–E10.
[124] Cortez MY, Torgan CE, Brozinick JT, and Ivy JL. 1991. Insulin resistance of obese Zucker rats exercise trained at two different intensities. *Am J Physiol Endocrinol Metab* 261: E613–E619.
[125] Eriksson JG. 1999. Exercise and the treatment of type 2 diabetes mellitus. An update. *Sports Med* 27: 381–391.
[126] Steinberg, G.R., et al. 2006. Tumor necrosis factor-α induced skeletal muscle insulin resistance involves suppression of AMP-kinase signaling. Cell Metabolism 4: 465-474.
[127] Bastard, J. P., Jardel, C., et al. 2000. Elevated levels of interleukin 6 are reduced in serum and
subcutaneous adipose tissue of obese women after weight loss. J Clin Endocrinol Metab 85, 3338–3342.
[128] Mohamed-Ali, V., Goodrick, S., et al. 1997. *J. Clin. Endocrinol. Metab.* **82:** 4196–4200.
[129] Straub, R. H., Hense, H. W., et al. 2000. *J. Clin. Endocrinol. Metab.* **85:** 1340–1344.
[130] Fernandez-Real, J. M., Vayreda, M., et al. 2001. *J. Clin. Endocrinol. Metab.* **86,** 1154–1159.
[131] Muller, S., Martin, S., et al. 2002. *Diabetologia* **45,** 805–812
[132] Kado, S., Nagase, T., and Nagata, N. 1999. *Acta Diabetol.* **36,:**67–72
[133] Pickup, J. C., Mattock, M. B., Chusney, G. D., and Burt, D. 1997. *Diabetologia* **40:** 1286–1292
[134] Pradhan, A. D., Manson, J. E., et al. 2001. *J. Am. Med. Assoc.* **286,** 327–334.
[135] Rotter, V., et al. 2003. Interleukin-6 (IL-6) induces insulin resistance in 3T3-L1 adipocytes and is, like IL-8 and tumor necrosis factor alpha, overexpressed in human fat cells from insulin resistant subjects. Journal of Biological Chemistry 278(46): 45777-45784.
[136] Emanueli, B., P., *et al*. 2000. SOCS-3 is an insulin-induced negative regulator of insulin signaling. J. Biol. Chem. **275:** 15985–15991.
[137] Ueki, K., et al. 2004. Suppressor of cytokine signaling 1 (SOCS-1) and SOCS-3 cause insulin resistance through inhibition of tyrosine phosphorylation of insulin receptor substrate proteins by discrete mechanisms. Mol. Cell. Biol. **24:** 5434–5446.
[138] Senn, J. J., Klover, P. J., Nowak, I. A., & Mooney, R. A. 2002. Interleukin-6 induces cellular insulin resistance in hepatocytes. Diabetes 51: 3391– 3399.

[139] Greenberg, A. S., Nordan, R. P., et al. 1992. Interleukin 6 reduces lipoprotein lipase activity in adipose tissue of mice in vivo and in 3T3-L1 adipocytes: a possible role for interleukin 6 in cancer cachexia. Cancer Res Cancer Res 52: 4113– 4116.
[140] Liu, E., Kitajima, S., et al. 2005a. High lipoprotein lipase activity increases insulin sensitivity in transgenic rabbits. Metabolism 54,:132– 138.
[141] Liu, H. B., et al. 2005b. Thiazolidinediones inhibit TNFalpha induction of PAI-1 independent
of PPARgamma activation. Biochem Biophys Res Commun 334: 30–37.
[142] Otarod, J. K., & Goldberg, I. J. 2004. Lipoprotein lipase and its role in regulation of plasma lipoproteins and cardiac risk. Curr Atheroscler Rep 6: 335– 342.
[143] Matias, I et al. 2006. "Regulation, function, and dysregulation of endocannabinoids in models of adipose and beta pancreatic cells and in obesity and hyperglycemia." Journal of Clinical Endocrinology and Metabolism e-Pub ahead of print-May 9, 2006.
[144] Saoirse, E., et al. 2005. Novel time-dependent vascular actions of Δ9-tetrahydrocannabinol mediated by peroxisome proliferators activated receptor gamma. Biochemical and Biophysical Research Communications 327: 824-831.
[145] Burstein, S. 2005. PPARγ: a nuclear receptor with affinity for cannabinoids. Life Sciences 77: 1674-1684.
[146] Roden, M. 2004. How free fatty acids inhibit glucose utilization in human skeletal muscle. News Physiol Sci 19, 92– 96.
[147] Imoto, H., Imamiya, E., et al. 2002. Studies on non-thiazolidinedione antidiabetic agents: 1. Discovery
of novel oxyiminoacetic acid derivatives. Chem Pharm Bull (Tokyo) 50: 1349–1357.
[148] Imoto, H., et al. 2003. Studies on nonthiazolidinedione antidiabetic agents: 2. Novel oxyiminoalkanoic acid derivatives as potent glucose and lipid lowering agents. Chem Pharm Bull (Tokyo) 51, 138– 151.
[149] Lehmann, J. M., Moore, L. B., et al. 1995. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). J Biol Chem 270, 12953– 12956.
[150] Willson, T. M., Cobb, J. E., et al. 1996. The structure-activity relationship between peroxisome proliferator-activated receptor gamma agonism and the antihyperglycemic activity of thiazolidinediones. J Med Chem 39, 665–668.
[151] Fujita, T., Sugiyama, Y., et al. 1983. Reduction of insulin resistance in obese and/or diabetic animals by 5-[4-(1-methylcyclohexylmethoxy)benzyl]-thiazolidine-2,4- dione (ADD-3878, U-63,287, ciglitazone), a new antidiabetic agent. Diabetes 32, 804–810.
[152] Elbrecht, A., Chen, Y., et al. 1996. Molecular cloning, expression and characterization of human peroxisome proliferator activated receptors gamma 1 and gamma 2. Biochem Biophys Res Commun 224, 431– 437.
[153] Tontonoz, P., Hu, E., & Spiegelman, B. M. 1994. Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid-activated transcription factor. Cell 79, 1147– 1156.
[154] Gregoire, F. M., Smas, C. M., & Sul, H. S. 1998. Understanding adipocyte differentiation. Physiol Rev 78, 783– 809.
[155] Tontonoz, P., Hu, E., Devine, J., Beale, E. G., & Spiegelman, B. M. 1995. PPAR gamma 2 regulates adipose expression of the phosphoenolpyruvate carboxykinase gene. Mol Cell Biol 15, 351– 357.

[156] Berger, J., & Moller, D. E. 2002. The mechanisms of action of PPARs. Annu Rev Med 53, 409– 435.
[157] Guo, L. and Tabrizchi, R. 2005. Peroxisome-proliferator activated receptor gamma as a drug target in the pathogenesis of insulin resistance. Pharmacology & Therapeutics.
[158] Kola, B. et al. 2005. Cannabinoids and ghrelin have both central and peripheral metabolic and cardiac effects via AMP-activated protein kinase. J. Biol. Chem. 280, 25196–25201.
[159] Smith, S.R., et al. 2000. Effects of cannabinoid receptor agonist and antagonist ligands on production of inflammatory cytokines and anti-inflammatory interleukin-10 in endotoxemic mice. J. Pharmacol. Exp. Ther. 293, 136– 150.
[160] Klein, T.W., Newton, C.A., et al. 1985. The effect of delta-9-tetrahydrocannabinol and 11-hydroxy-delta-9-tetrahydrocannabinol on T-lymphocyte and B-lymphocyte mitogen responses. J. Immunopharmacol. 7, 451– 466.
[161] Newton, C.A., Klein, T.W., Friedman, H. 1994. Secondary immunity to Legionella pneumophila and Th1 activity are suppressed by delta-9- tetrahydrocannabinol injection. Infect. Immun. 62, 4015–4020.
[162] Croxford, J.L. and Yamamura, T. 2005. Cannabinoids and the immune system: Potential for the treatment of inflammatory diseases. Journal of Neuroimmunology 166: 3-18.
[163] Zheng, Z.M., Specter, S., Friedman, H.,. 1992. Inhibition by delta-9-tetrahydrocannabinol of tumor necrosis factor alpha production by mouse and human macrophages. Int. J. Immunopharmacol. 14,
1445– 1452.
[164] Kusher, D.I., Dawson, L.O., Taylor, A.C., Djeu, J.Y.. 1994. Effect of the psychoactive metabolite of marijuana, delta 9-tetrahydrocannabinol (THC), on the synthesis of tumor necrosis factor by human large
granular lymphocytes. Cell. Immunol. 154, 99– 108.
[165] Srivastava, M.D., Srivastava, B.I., Brouhard, B. 1998. Delta 9 tetrahydrocannabinol and cannabidiol alter cytokine production by human immune cells. Immunopharmacology 40, 179– 185.
[166] Watzl, B., Scuderi, P.,Watson, R.R. 1991. Marijuana components stimulate human peripheral blood mononuclear cell secretion of interferongamma and suppress interleukin-1 alpha in vitro. Int. J. Immunopharmacol. 13, 1091– 1097.
[167] Puffenbarger, R.A., Boothe, A.C., Cabral, G.A.. 2000. Cannabinoids inhibit LPS-inducible cytokine mRNA expression in rat microglial cells. Glia 29, 58– 69.
[168] Ayalasomayajula, S.P. and Kompella, U.B. 2003. *Eur. J. Pharmacol.,* **458,** 283-289.
[169] Joussen, A.M.; Poulaki, V.; Mitsiades, N., et al. 2002. *FASEB J.,* **16,** 438-440.
[170] Cheng, T.; Cao, W.; Wen, R.; Steinberg, R.H. and LaVail, M.M. 1998. *Invest. Ophthalmol. Vis. Sci.,* **39,** 581-591.
[171] Nie, D.; Lamberti, M., et al. 2000. *Biochem. Biophys. Res. Commun.,* **267:** 245-251.
[172] Hata, Y.; Clermont, A., et al. 2000. *J. Clin. Invest.,* **106,** 541-550.
[173] Tsujii, M.; Kawano, S.; Tsuji, S.; Sawaoka, H.; Hori, M. and DuBois, R.N. 1998. *Cell,* **93,** 705-716.
[174] Jones, M.K.; Wang, H.; Peskar, B.M.; Levin, E.; Itani, R.M.;
Sarfeh, I.J. and Tarnawski, A.S. 1999. *Nat. Med.,* **5,** 1418-1423.

[175] Sone, H.; Kawakami, Y.; Segawa, T., et al. 1999. *Life Sci.,* **65,** 2573-2580.
[176] Sone, H.; Okuda, Y.; Kawakami, Y. and Yamashita, K. 1996. *Life Sci.,* **58,** 239-243.
[177] Qaum T, Xu Q, Joussen AM, et al. 2001. VEGF-initiated blood-retinal barrier breakdown in early diabetes. Invest Ophthalmol Vis Sci 42:2408–2413
[178] El-Remessy AB, Behzadian MA, et al. 2003. Experimental diabetes causes breakdown of the blood-retina barrier by a mechanism involving tyrosine nitration and increases in expression of vascular endothelial growth factor and urokinase plasminogen activator receptor. Am J Pathol 162:1995–2004
[179] Joussen AM, Poulaki V, et al. 2002. Nonsteroidal anti-inflammatory drugs prevent early diabetic retinopathy via TNF-alpha suppression. FASEB J 16:438–440
[180] Honjo M, Tanihara H, Nishijima K, et al. 2002. Statin inhibits leukocyte-endothelial interaction and prevents neuronal death induced by ischemia-reperfusion injury in the rat retina. Arch Ophthalmol 120:1707–1713.
[181] El-Remessy, A.B., et al. 2006. Neuroprotective and blood-retinal barrier preserving effects of cannabidiol in experimental diabetes. American Journal of Pathology 168(1): 235-244.
[182] Bartoli, M., Platt, D.H., Lemtalsi, T., **El-Remessy, A.B**., Marrero, M. and Caldwell, R.B. High glucose-induced oxidative stress modifies VEGF-dependent STAT3 activation. Free Rad. Biol. Med,in revision
[183] Caldwell, R.B., et al. 2005. Vascular endothelial growth factor and diabetic retinopathy: role of oxidative stress. Current Drug Targets 6: 511-524.
[184] Platt, D.H. Bartoli, M.,et al. 2005. Peroxynitrite-mediated activation of VEGF Transcription in Vascular cells via signal transducer and activator of transcription-3. Free Rad. Biol. Med, 39: 1353-1361.
[185] He, H.; Venema, V.J.; Gu, X.; Venema, R.C., et al. 1999. *J. Biol. Chem.,* **274,** 25130-25135.
[186] Niu, G.; Wright, K.L.; Huang, M.; Song, L.; Haura, E.; Turkson, J.; Zhang, S.; Wang, T.; Sinibaldi, D.; Coppola, D., et al. 2002. *Oncogene,* **21,** 2000-2008.
[187] Ellis, E.A.; Grant, M.B., et al. 1998. *Free Radic. Biol. Med.,* **24,** 111-120.
[188] Ellis, E.A.; Guberski, D.L., et al. 2000. *Free Radic. Biol. Med.,* **28,** 91-101.
[189] Kuroki, M.; Voest, E.E., et al. 1996. *J. Clin. Invest.,* **98,** 1667-1675.
[190] Obrosova, I.G.; Minchenko, A.G., et al. 2001. *Diabetologia,* **44,** 1102-1110.
[191] Brownlee, M. 2001. Biochemistry and molecular biology of diabetic complications*Nature,* **414,** 813-820.
[192] Lee, H.B.; Yu, M.R.; Yang, Y.; Jiang, Z. and Ha, H. 2003. *J. Am. Soc. Nephrol.,* **14,** S241-245.
[193] Nakagami H, Morishita R, Yamamoto K, et al. 2001. Phosphorylation of p38 mitogen-activated protein kinase downstream of baxcaspase- 3 pathway leads to cell death induced by high D-glucose in human endothelial cells. Diabetes 50:1472–1481
[194] El-Remessy AB, Bartoli M, Platt DH, Fulton D, Caldwell RB. 2005. Oxidative stress inactivates VEGF survival signaling in retinal endothelial cells via PI 3-kinase tyrosine nitration. J Cell Sci 118:243–252.
[195] Igarashi M, Wakasaki H, Takahara N, et al. 1999. Glucose or diabetes activates p38 mitogen-activated protein kinase via different pathways. J Clin Invest 103:185–195

[196] Purves T, Middlemas A, Agthong S, et al. 2001. A role for mitogen-activated protein kinases in the
etiology of diabetic neuropathy. FASEB J 15:2508–2514
[197] Kikuchi M, Tenneti L, Lipton SA. 2000. Role of p38 mitogen-activated protein kinase in axotomy-induced apoptosis of rat retinal ganglion cells. J Neurosci 20:5037–5044
[198] Miller, J.W.; Adamis, A.P. and Aiello, L.P. 1997. *Diabetes Metab Rev,* **13,** 37-50.
[199] Duh, E. and Aiello, L.P. 1999. *Diabetes,* **48,** 1899-1906.
[200] Gilbert, R.E.; Vranes, D., et al. 1998. *Lab. Invest.,* **78,** 1017-1027.
[201] Hammes, H.P.; Lin, J., et al. 1998. *Diabetes,* **47,** 401-406.
[202] Barber, A.J. 2003. *Prog Neuropsychopharmacol Biol Psychiatry,* **27,** 283-290.
[203] Martin, P.M.; Roon, P., et al. 2004. *Invest. Ophthalmol. Vis. Sci.,* **45,** 3330-3336.
[204] Mohr, S.; Xi, X.; Tang, J. and Kern, T.S. 2002. *Diabetes,* **51,** 1172-1179.
[205] Ning, X.; Baoyu, Q.; Yuzhen, L.; Shuli, S.; Reed, E. and Li, Q.Q. 2004. *Int. J. Mol. Med.,* **13,** 87-92.
[206] Davis MD, Kern TS, Rand LI. Diabetic retinopathy. In *International Textbook Of Diabetes Mellitus* (2nd edn), vol. 2. Alberti KGMM, Zimmet P, DeFronzo RA (eds). Wiley: Chichester, 1997; 1413–1446.
[207] DeLaCruz, J.P., et al. 2004. Pharmacological approach to diabetic retinopathy. Diabetes/Metabolism Research and Reviews 20: 91-113.
[208] Davis MD. 1992. Diabetic retinopathy. A clinical overview. *Diabetes Care* **15**: 1844–1874.
[209] Ferris FL III, Patz A. 1984. Macular edema. A complication of diabetic retinopathy. *Surv Ophthalmol* **28**(Suppl. 1): 452–461.
[210] Adamis, A.P.; Miller, J.W., et al. 1994. *Am. J. Ophthalmol.,* **118,** 445-450.
[211] Aiello, L.P.; Avery, R.L., et al. 1994. *New Eng. J. Med.,* **331,** 1480-1487.
[212] Takeda, M.; Mori, F., et al. 2001. *Diabetologia,* **44,** 1043-1050.
[213] Senger, D.R.; Galli, S.J.; Dvorak, A.M.; Perruzzi, C.A.; Harvey, V.S. and Dvorak, H.F. 1983. *Science,* **219,** 983-985.
[214] Feng, Y.; Venema, V.J.; Venema, R.C.; Tsai, N.; Behzadian, M.A. and Caldwell, R.B. 1999. *Invest. Ophthalmol. Vis. Sci.,* **40,** 157-167.
[215] Behzadian, M.A.; Windsor, L.J.; Ghaly, N.; Liou, G.; Tsai, N.T. and Caldwell, R.B. 2003. *FASEB J.,* **19,** 19.
[216]ETDRS Report Number 12. 1991. Early Treatment Diabetic Retinopathy Study Research Group. Fundus photographic risk factors for progression of diabetic retinopathy: . *Ophthalmology* **98**(Suppl. 5): 823–833.
[217] Moss SE, Klein R, Klein BE. Ocular factors in the incidence and progression of diabetic retinopathy. *Ophthalmology* 1994; **101**: 77–83.
[218] Behzadian, M.A.; Wang, X.L., et al. 2001. *Invest. Ophthalmol. Vis. Sci.,* **42,** 853-859.
[219] Ishida, S.; Usui, T.; Yamashiro, K.; Kaji, Y., et al. 2003. *J. Exp. Med.,* **198,** 483-489.
[220] Asahara, T.; Murohara, T.; Sullivan, A.; Silver, M.; van der Zee, R.; Li, T.; Witzenbichler, B., et al. 1997. *Science,* **275,** 964-967.
[221] Manabe S, Lipton SA. 2003. Divergent NMDA signals leading to proapoptotic and antiapoptotic pathways in the rat retina. Invest Ophthalmol Vis Sci 2003, 44:385–392

[222] Poulaki V, Qin W, Joussen AM, Hurlbut P, et al. 2002. Acute intensive insulin therapy exacerbates
diabetic blood-retinal barrier breakdown via hypoxia-inducible factor-1alpha and VEGF. J Clin Invest 109:805–815.
211 Grant, M.B.; May, W.S.; Caballero, S.; Brown, G., et al. 2002. *Nat. Med.,* **8,** 607-612.
[224] Levy, A.P.; Levy, N.S. and Goldberg, M.A. 1996. *J. Biol. Chem.,* **271,** 2746-2753.
[225] Ushio-Fukai, M.; Tang, Y.; Fukai, T.; Dikalov, S.I.; Ma, Y.; Fujimoto, M.; Quinn, M.T, et al. 2002. *Circ. Res.,* **91,** 1160-1167.
[226] Ryuto, M.; Ono, M.; Izumi, H., et al. 1996. *J. Biol. Chem.,* **271,** 28220-28228.
[227] Li, J.; Perrella, M.A.; Tsai, J.C., et al. 1995. *J. Biol. Chem.,* **270,** 308-312.
[228] Pertovaara, L.; Kaipainen, A., et al. 1994. *J. Biol. Chem.,* **269,** 6271-6274
[229] Goad, D.L.; Rubin, J.; Wang, H.; Tashjian, A.H., Jr. and Patterson, C. 1996. *Endocrinology,* **137,** 2262-2268.
[230] Cohen, T.; Nahari, D.; Cerem, L.W.; Neufeld, G. and Levi, B.Z. 1996. *J. Biol. Chem.,* **271,** 736-741.
[231] Colavitti, R.; Pani, G.; Bedogni, B., et al. 2002. *J. Biol. Chem.,* **277,** 3101-3108.
[232] Morbidelli, L.; Chang, C.H., et al. 1996. *Am. J. Physiol.,* **270,** H411-415.
[233] Ziche, M.; Morbidelli, L., et al. 1997. *J. Clin. Invest.,* **99,** 2625-2634.
[234] Gabbay KH. The sorbitol pathway and the complications of
diabetes. N Engl J Med. 1973. 288: 831–836.
[235] Williamson JR, Chang K, Frangos M, *et al*. 1993. Perspectives in Diabetes. Hyperglycemic pseudohypoxia and diabetic complications. *Diabetes* **42**: 801–813.
[236] Greene DA, Stevens MJ. The sorbitol-osmotic and sorbitol redox hypotheses. 1996. *Diabetes Mellitus*. Lippincott Raven: Philadelphia, 1996; 801–809.
[237] King GL, Brownlee M. 1996. The cellular and molecular mechanisms of diabetic complications. *Endocrinol Metab Clin North Am* **25**: 255–270.
[238] Cameron NE, Cotter MA. 1992. Impaired contraction and relaxation in aorta from streptozotocin-diabetic rats: role of polyol pathway. *Diabetologia* 1992; **35**: 1011–1019
[239] Pugliese G, Tilton RG, Williamson JR. 1991. Glucose-induced metabolic imbalances in the pathogenesis of diabetic vascular disease. *Diabetes Metab Rev* 1991; **7**: 35–59.
[240] Cohen RA. Endothelial dysfunction in diabetic vascular disease. 1997. *Mediographia* **87**: 31–38.
[241] Koya D, King GL. Protein kinase C activation and the development of diabetic complications. 1998. *Diabetes* **47**: 859–866.
[242] Vlassara H. 1997. Recent progress in advanced glycation end products and diabetic complications. *Diabetes* **46**(Suppl. 2): S19–S25.
[243] Newton AC. Regulation of protein kinase C. 1997. *Curr Opin Cell Biol* **9**: 161–167.
[244] Johannes FJ, Prestle J, Eis S, *et al*. 1994. PKCu is a novel, atypical member of the protein kinase C family. *J Biol Chem*1994; **269**: 6140–6148.
[245] Wolff SP, Crabbe MJC, Thornalley PJ. 1984. The autoxidation of glyceraldehyde and other simple monosaccharides. *Experientia* **40**: 244–248.

[246] Asnaghi V, Gerhardinger C, Hoehn T, et al. 2003. A role for the polyol pathway in the early neuroretinal apoptosis and glial changes induced by diabetes in the rat. Diabetes 52:506–511

[247] Barber AJ, Lieth E, et al. 1998. Neural apoptosis in the retina during experimental and human diabetes. Early onset and effect of insulin. J Clin Invest 102:783–791

[248] Hornalley PJ, Wolff SP, Crabbe MJ, *et al*. 1984. The oxidation of oxyhaemoglobin by glyceraldehyde and other simple monosaccharides. *Biochem J* 1984; **217**: 615–622.

[249] Dreyer EB, Zurakowski D, Schumer RA, Podos SM, Lipton SA. 1996. Elevated glutamate levels in the vitreous body of humans and monkeys with glaucoma. Arch Ophthalmol 114:299–305.

[250] Nishikawa T, Edelstein D, Du XL, *et al*. 2000. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* **404**: 787–790.

[251] Takagi Y, Kashiwagi A, Tanaka Y, *et al*. 1995. Significance of fructose induced protein oxidation and formation of advanced glycation end product. *J Diabetes Complications* **9**: 87–89.

[252] Stitt AW, Li YM, Gardiner TA, *et al*. 1997. Advanced glycation end products (AGEs) co-localize with AGE receptors in the retinal vasculature of diabetic and of AGE-infused rats. *Am J Pathol* **150**: 523–531.

[253] Stitt AW, He C, Vlassara H. 1999. Characterization of the advanced glycation end-product receptor complex in human vascular endothelial cells. *Biochem Biophys Res Commun* **256**: 549–556.

[254] Mohamed AK, Bierhaus A, Schiekofer S, *et al*. 1999. The role of oxidative stress and NF-kappa ß activation in late diabetic complications. *Biofactors* 1999; **10**: 157–167.

[255] Yamagishi SI, Yonekura H, Yamamoto Y, *et al*. 1997. Advanced glycation end products driven angiogenesis in vitro. Induction of the growth and tube formation of human microvascular endothelial cells through autocrine vascular endothelial growth factor. *J Biol Chem* **272**: 8723–8730.

[256] Barrett ML, Gordon D, Evans FJ. 1985. Isolation from Cannabis sativa L. of cannflavin—a novel inhibitor of prostaglandin production. Biochem Pharmacol 34:2019–2024.

[257] Barrett ML, Scutt AM, Evans FJ. 1986. Cannflavin A and B, prenylated flavones from Cannabis sativa L. Experientia 42:452–453

[258] Burstein S, Varanelli C, Slade LT. 1975. Prostaglandins and Cannabis III. Inhibition of biosynthesis by essential oil components of marihuana. Biochem Pharmacol 24: 1053–1058.

[259] Burstein S, Taylor P, Turner C, El-Feraly FS. 1976. Prostaglandins and Cannabis V. Identification of pvinylphenol as a potent inhibitor of prostaglandin synthesis. Biochem Pharmacol 25:2003–2009.

[260] Sofia RD, Nalepa SD, Harakal JJ, Vassar HB. 1973. Antiedema and analgesic properties of D9-tetrahydrocannabinol (THC). J Pharmacol Exp Therap 186:646–655.

[261] Sofia RD, Nalepa SD, Vassar HB, Knobloch LC. 1974. Comparative anti-phlogistic activity of D9-tetrahydrocannabinol, hydrocortisone, and aspirin in various rat paw edema models. Life Sci 15:251–260.

[262] Wirth PW, Watson ES, ElSohly M, Turner CE, Murphy JC. 1980. Anti-inflammatory properties of cannabichromene. Life Sci 26:1991–1995.
[263] Turner CE, ElSohly M. 1981. Biological activity of cannabichromene, its homologs and isomers. J Clin Pharmacol 21:283S–291S
[264] Spronck JW, Lutein M, Salemink A, Nugteren H. 1978. Inhibition of prostaglandin biosynthesis by derivatives of olivetol formed under pyrolysis of cannabidiol. Biochem Pharmacol 27:607–608.
[265] Blazquez, C.; Gonzalez-Feria, L., et al. 2004. *Cancer Res.,* **64,** 5617-5623.
[266] Blazquez, C.; Casanova, M.L., et al. 2003. *FASEB J.,* **17,** 529-531. Epub 2003 Jan 2002.
[267] Ciani E, Groneng L, Voltattorni M, et al. 1996. Inhibition of free radical production or free radical scavenging protects from the excitotoxic cell death mediated by glutamate in cultures of cerebellar granule neurons. Brain Res 728:1–6
[268] MacGregor DG, Higgins MJ, et al. 1996. Ascorbate attenuates the systemic kainite induced neurotoxicity in the rat hippocampus. Brain Res 727: 133–144
[269] Shen M, Thayer SA. 1998. Cannabinoid receptor agonists protect cultured rat hippocampal neurons from excitotoxicity. Mol Pharmacol 54:459–462
[270] Nagayama T, Sinor AD, et al. 1999. Cannabinoids and neuroprotection in global and focal cerebral ischemia and in neuronal cultures. J Neurosci 19:2987–2995
[271] Van der Stelt M, Veldhuis WB, et al. 2001. Neuroprotection by _9-tetrahydrocannabinol, the main
active compound in marijuana, against ouabain-induced in vivo excitotoxicity. J Neurosci 2001, 21:6475–6479.
[272] Hampson AJ, Grimald M, Axelrod J, Wink D. 1998. Cannabidiol and (_)_9-tetrahydrocannabinol are neuroprotective antioxidants. Proc Natl Acad Sci USA 95:8268–8273
[273] Marsicano G, Moosmann B, Hermann H, Lutz B, Behlt C. 2002. Neuroprotective properties of cannabinoids against oxidative stress: role of the cannabinoid receptor CB1. J Neurochem 80:448–456.
[274] El-Remessy, A.B., et al. 2003. Neuroprotective effect of tetrahydrocannabinol and cannabidiol in N-methyl-D-aspartate induced retinal neurotoxicity. American Journal of Pathology 163: 1997-2008.
[275] Hughes WF. 1991. Quantitation of ischemic damage in the rat retina. Exp Eye Res 53:573–582
[276] Beale R, Osborne NN. 1982. Localization of the Thy-1 antigen to the surface of rat retinal ganglion cells. Neurochem Int 4:587–595
[277] van der Stelt M, Veldhuis WB, van Haaften GW, et al. 2001. Exogenous anandamide protects rat brain against acute neuronal injury in vivo. J Neurosci 21:8765–8771
[278] Sinor AD, Irvin SM, Greenberg DA. 2000. Endocannabinoids protect cerebral cortical neurons from in vitro ischemia in rats. Neurosci Lett 278:157–160
[279] Zygmunt PM, Petersson J, et al. 1999. Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. Nature 400:452–457

[280] Maingret F, Patel AJ, Lazdunski M, Honore E. 2001. The endocannabinoid anandamide is a direct and selective blocker of the background K(_) channel TASK-1. EMBO J 2001, 20:47–54

[281] Valjent E, Pages C, Rogard M, Besson MJ, Maldonado R, Caboche J. 2001. Delta9-tetrahydrocannabinol-induced MAPK/ERK and Elk-1 activation in vivo depends on dopamineric transmission. Eur J Neurosci 14:342–352.

[282]Tiedeman JS, Shields MB, et al. 1981. Effect of synthetic cannabinoids on elevated intraocular pressure. Ophthalmology 88:270–277

[283] Beilin M, Neumann R, et al. 2000. Pharmacology of the intraocular pressure lowering effect of systemic dexanabinol (HU- 211), a non-psychotropic cannabinoid. J Ocul Pharmacol Ther 16:217–229

[284] Laine K, Jarvinen K, Mechoulam R, et al. 2002. Comparison of the enzymatic stability and intraocular pressure effects of 2-arachidonylglycerol and noladin ether, a novel putative endocannabinoid. Invest Ophthalmol Vis Sci 43:3216–3222.

[285] Panikashvill D, Simeonidou C, et al. 2001. An endogenous cannabinoid (2-AG) is neuroprotective after brain injury. Nature 413:527–531

[286] Song ZH, Slowey CA. 2000. Involvement of cannabinoid receptors in the intraocular pressure-lowering effects of WIN55212–2. J Pharmacol Exp Ther 292:136–139.

[287] Colwell, J. A. 1993. Vascular thrombosis in Type 2 diabetes mellitus. Diabetes 42: 8-11.

[288] Haffner, S.M, et al. 1998. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. New England Journal of Medicine 339: 229-234.

[289] Libby, P. 2002. Inflammation in Atherosclerosis. Nature 420: 868-874.

[290] Libby, P., et al. 2002. Inflammation and Atherosclerosis. Circulation 105: 1135-1143.

[291] Steiner, M., et al. 1994. Increased levels of soluble adhesion molecules in type 2 (non-insulin dependent) diabetes mellitus are independent of glycemic control. Thromb Haemost 72: 979-984.

[292] Cominacini, I., et al. 1997. E-selectin plasma concentration is influenced by glycemic control in NIDDM patients: possible role of oxidative stress. Diabetologia 40: 584-589.

[293] Marfella, R., et al. 2000. Circulating adhesion molecules in humans. Role of hyperglycemia and Hyperinsulinemia. Circulation 101: 2247-2251.

[294] Otsuki, M, et al. 1997. Circulating vascular cell adhesion molecule (VCAM-1) in atherosclerotic NIDDM patients. 46: 2096-2101.

[295] Cominacini, I., et al. 1995. Elevated levels of soluble E-selectin in patients with IDDM and NIDDM: relation to metabolic control. Diabetologia 38: 1122-1124.

[296] Fasching, P., et al. 1996. Elevated concentrations of circulating adhesion molecules and their association with microvascular in insulin-dependent diabetes mellitus. Journal of Clinical Endocrinology and Metabolism 81: 4313-4317.

[297] Gearing, A.J.H., et al. 1992. Soluble forms of vascular adhesion molecules, E-selectin, ICAM-1, and VCAM-1,: pathological significance. Annals New York Academy of Sciences 667: 324-331.

[298] Daugherty, A. and Rateri, D. L. 2002. T lumphocytes in Atherosclerosis: the yin-yang of Th1 and Th2 influence on lesion formation. Circ. Res. 90: 1039-1040.
[299] Moeller, F. and Nielson, L. B. 2003. Aortic recruitment of blood lymphocytes is most pronounced in early stages of lesion formation in apolipoprotein-E-deficient mice. Atherosclerosis 168: 49-56.
[300] Song, L., et al. 2001. Lymphocytes are important in early Atherosclerosis. Journal of Clinical Investigation 108: 151-259.
[301] Steffens, S., et al. 2005. Low dose oral cannabinoid therapy reduces progression of atherosclerosis in mice. Nature 434: 782-786.
[302] Steffens, S. and Mach, F. 2006. Cannabinoid receptors in atherosclerosis. Current Opinion in Lipidology 17: 1-8.
[303] Sarcerdote, P., et al. 2005. The nonpsychoactive component of marijuana cannabidiol modulates chemotaxis and IL-10 and IL-12 production of murine macrophages both in vitro and in vivo. Journal of Neuroimmunology 159: 97-105.
[304] Rinaldi-Carmona, M., et al. 1998. SR144528, the first potent and selective antagonist of the CB2 cannabinoid receptor. Journal of Pharmacology and Experimental Therapeutics 284: 644-650.
[305] Joseph, J., et al. 2004. Anandamide is an endogenous inhibitor for the migration of tumor cells and T lymphocytes. Cancer Immunology and Immunotherapy 53: 723-728.
[306] Malfait, A.M., et al. 2000. The nonpsychoactive cannabis constituent cannabidiol is an oral anti-arthritic therapeutic in murine collagen-induced arthritis. Proceedings of the National Academy of Sciences97: 9561-9566.
[307] Sulcova, E., et al. 1998. Biphasic effects of anandamide. Pharmacol. Biochem. Behavior 59: 347-352.
[308] Tontonoz, P., et al. 1998. PPARγ promotes monocyte/macrophage differentiation and uptake of oxidized LDL. Cell 93: 241-252.
[309] Nagy, L., et al. 1998. Oxidized LDL regulates macrophage gene expression through ligand activation of PPARγ. Cell 293: 229-240.
[310] Ricote, M., et al. 1998. The peroxisome proliferators activated receptor gamma is a negative regulator of macrophage activation. Nature 391: 79-82.
[311] Jiang, C., et al. 1998. PPARγ agonists inhibit production of monocyte inflammatory cytokines. Nature 391: 82-86.
[312] Plutzky, J. 2001. Peroxisome proliferators activated receptors in endothelial cell biology. Current Opinion in Lipidology 12: 511-518.
[313] Yang, X. Y., et al. 2000. Activation of human T lymphocytes is inhibited by peroxisome proliferators activated receptor γ (PPARγ) agonists. Co-association with transcription factor NFAT. Journal of Biological Chemistry 275: 4541-4544.
[314] Yuan, M., et al. 2002. Delta 9-tetrahydrocannabinol regulates Th1/Th2 cytokine balance in activated human T cells. Journal of Neuroimmunology 133: 124-131.
[315] Zygmunt, P.N., et al. 1999. vanilloid receptors on sensory nerves mediate the vasodilator effects of anandamide. Nature 400: 452-457.
[316] Watanabe K, et al. 1996. Inhibition of anandamide amidase activity in mouse brain microsomes by cannabinoids. Biol Pharm Bull 1996; 19:1109–1111.

[317] Bisogno T, et al. 2001. Molecular targets for cannabidiol and its synthetic analogues: effect of vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. Br J Pharmacology 134: 845–852.
[318] Benowitz NL, Jones RT. 1981. Cardiovascular and metabolic considerations in prolonged cannabinoid administration in man. J Clin Pharmacol 21: 214S–223S.
[319] Akins D, Awdeh MR. 1981. Marijuana and second-degree AV block. South Med J 74: 371–376.
320 Sullivan, S.E., et al. 2005. Novel time-dependent vascular actions of delta 9-tetrahydrocannabinol are mediated by peroxisome proliferators activated receptor gamma. Biochemistry Biophysics Research Communications 337: 824-831.
[321] Bishop-Bailey, D. 2000. Peroxisome proliferators activated recptors in the cardiovascular system. British Journal of Pharmacology 129: 823-834.
[322] Hsueh, W.A, et al. 2004. Peroxisome proliferators activated receptor gamma: implications for cardiovascular disease. Hypertension 43: 297-305.
[323] Tsukamoto, T., et al. 2004. Thiazolidinediones increase arachidonic acid release and subsequent prostanoids production in a peroxisome proliferators activated receptor gamma independent manner. Prostaglandins and Other Lipid mediators 73: 191-213.
[324] Krylatov AV, Bernatskaia NA, et al. 2002. Increase of the heart arrhythmogenic resistance and decrease of the myocardial necrosis zone during activation of cannabinoid receptors. Ross Fiziol Zh Im I M Sechenova 88: 560–567.
[325] Mishima K, et al. 2004. Effects of hypothermia and hyperthermia on attentional and spatial learning deficits following neonatal hypoxia-ischemic insult in rats. Behav Brain Res 151:209–217.
[326] Nava F, Carta G, et al. 2000. Permissive role of dopamine D(2) receptors in the hypothermia induced by delta(9)-tetrahydrocannabinol in rats. Pharmacol Biochem Behav 2000; 66:183–187.
[327] Hampson AJ, et al. 2002. Neuroprotective antioxidants from marijuana. Ann NY Acad Sci 899:274–282.
[328] Hayakawa, K., et al. 2004. Cannabidiol prevents infarction via the non-CB1 receptor mechanism. Neuropharmacology and Neurotoxicology 15: 2381-2385.
[329] Schmist, Y., et al. 2006. delta 9-tetrahydrocannabinol protects cardiac cells from hypoxia via CB2 receptor activation and nitric oxide production. Molecular and Cellular biochemistry 283: 73-85.
[330] Bolli R. 2001. Cardioprotective function of inducible nitric oxide synthase and role of nitric oxide in myocardial ischemia and pre-conditioning: An overview of a decade of research. J Mol Cell Cardiol 33: 1897–1918.
[331] Palmer RM, et al. 1987. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 327: 524–526.
[332] Joyeux M, et al. 2002. Endocannabinoids are implicated in the infarct size-reducing effect conferred by heat stress pre-conditioning in isolated rat hearts. Cardiovasc Res 55: 619–625.
[333] Samdani, A.F., et al. 1997. Nitric oxide synthase in models of focal ischemia. Stroke 28: 1283-1288.
[334] Hillard, C.J., et al. 1999. Effects of Cb1 cannabinoid receptor activation on cerebellar granule cell nitric oxide synthase activity. FEBS Letters 459: 277-281.

[335] Azad, S.C., et al. 2001. Differential role of the nitric oxide pathway on delta 9-THC induced central nervous system effects in the mouse. European Journal of Neuroscience 13: 561-568.
[336] Deutsch, D.G., et al. 1997. Production and physiological actions of anandamide in the vasculature of the rat kidney. Journal of Clinical Investigation 100: 1538-1546.
[337] Liang Du, X., et al. 2001. Hyperglycemia inhibits endothelial nitric oxide synthase activity by post-translational modification at the Akt site. Journal of Clinical Investigation 108: 1341-1348.
[338] Makimattila, S., et al. 1996. Chronic hyperglycemia impairs endothelial function and insulin sensitivity via different mechanisms in insulin dependent diabetes mellitus. *Circulation.* **94**:1276–1282.
[339] Luscher, T.F., et al. 1993. Endothelial dysfunction and coronary artery disease. *Annu. Rev. Med.* **44**:395–418.
[340] Schafers, M., et al. 2004. Cyclooxygenase inhibition in nerve-injury- and TNF-induced hyperalgesia in the rat. Exp. Neurol. 185, 160–168.
[341] A.R. Jadat, D. Carroll, et al. 1992. Morphine responsiveness of chronic pain: double-blind randomized crossover study with patient-controlled analgesia, Lancet 339: 1367–1371.
[342] Flood, J., Mooradian, A. & Morley, J. 1990. Characteristics of learning and memory in streptozotocin-induced diabetic mice. Diabetes, 39, 1391–1398.
[343] McCall, A. 1992. The impact of diabetes on the CNS. Diabetes, 41: 557–570.
[344] Biessels, G.J., Kappelle, A., et al. 1994. Cerebral function in diabetes mellitus. Diabetologia, 37, 643–650.
[345] Mankovsky, B. 1997. Cerebrovascular disorders in patients with diabetes mellitus. J. Diab. Complicat., 10, 228–242.
[346] Helgason, C. 1998. Blood glucose and stroke. Stroke, 19, 1049–1053.
[347] Ott, A., Stolk, R., et al. 1999. Diabetes mellitus and the risk of dementia: the Rotterdam study. Neurology, 58, 1937–1941.
[348] Stewart, R. & Liolitsa, D. 1999. Type 2 diabetes mellitus, cognitive impairment and dementia. Diabet. Med., 16, 93–112.
[349] Lustman, P., Griffith, L., Gavard, J. & Clouse, R. 1992. Depression in adults with diabetes. Diabetes Care, 15, 1631–1639.
[350] McEwen, B., Magarin˜ os, A. & Reagan, L. 2002. Studies of hormone action in the hippocampal formation. Possible relevance to depression and diabetes. J. Psychosom. Res., 53, 883–890.
[351] Katon, W., von Korff, M., et al. 2004. Behavioral and clinical factors associated with depression among individuals with diabetes. Diabetes Care, 27, 914.
[352] Tomiyama M, et al. 2005. Upregulation of mRNAs coding for AMPA and NMDA receptor subunits and metabotropic glutamate receptors in the dorsal horn of the spinal cord in a rat model of diabetes mellitus. Brain Res Mol Brain Res 136: 275–281.
[353] Martin L. J., Al-Abdulla N. A., et al. 1998. Neurodegeneration in excitotoxicity, global cerebral ischemia, and target deprivation: a perspective on the contributions of apoptosis and necrosis. Brain Res. Bull. **46:** 281–309.
[354] Zipfel G. J., Babcock D. J.,et al. 2000. Neuronal apoptosis after CNS injury: the roles of glutamate and calcium. J. Neurotrauma **17:** 857–869.

[355] C.D. Malis, J.V. Bonventre. 1986. Mechanism of calcium potentiation of oxygen free radical injury to renal mitochondria. A model for postischemic and toxic mitochondrial damage, J. Biol. Chem. 261: 14201–14208.
[356] O. Vergun, A.I. Sobolevsky,et al. 2001. Exploration of the role of reactive oxygen species in glutamate neurotoxicity in rat hippocampal neurons in culture, J. Physiol. 531: 147–163.
[357] Garthwaite, G, and Garthwaite, J. 1986. Neurotoxicity of excitatory amino acid receptor agonists in rat cerebellar slices: dependence on calcium concentration, Neurosci. Lett. 66: 193–198.
[358] C.M. Luetjens, N.T. Bui, et al. 2000. Delayed mitochondrial dysfunction in excitotoxic neuron death: cytochrome c release and a secondary increase in superoxide production, J. Neurosci. 20: 5715–5723.
[359] Zhang W., Khanna P., et al. 1997. Diabetes induced apoptosis in rat kidney. Biochem. Mol. Med. **61:** 58–62.
[360] Baumgartner-Parzer S. M., et al. 1995. High-glucose-triggered apoptosis in cultural endothelial cells. Diabetes **44:** 1323–1327.
[361] Srinivasan S., Stevens M. J., et al. 1998. Serum from patients with type 2 diabetes with neuropathy induces complement-independent, calcium-dependent apoptosis in cultured neuronal cells. J. Clin. Invest. **102:** 1454–1462.
[362] Pittinger G. L., Lin D., et al. 1997. The apoptotic death of neuroblastoma cells caused by serum from patients with insulin-dependent diabetes and neuropathy may be Fas mediated. J. Neuroimmunol. **76:** 153–160.
[363] Srinivasan S., et al. 2000. Diabetic peripheral neuropathy: evidence for apoptosis and associated mitochondrial dysfunction. Diabetes **49:** 1932–1938.
[364] Russell J. W., et al. 1999. Neurons undergo apoptosis in 2460 A. A. F. Sima New understandings in diabetic neuropathy animal and cell culture models of diabetes. Neurobiol. Dis. **6:** 347–363.
[365] Schmeichel A. M., et al. 2003. Oxidative injury and apoptosis of dorsal root ganglion neurons in chronic experimental diabetic neuropathy. Diabetes **52:** 162–171.
[366] Crompton M. 1999. The mitochondrial permeability transition pore and its role in cell death. Biochem. J. **341:** 233–249.
[367] R.W. Chen, Z.H. Qin, et al. 2003. Regulation of c-Jun N-terminal kinase, p38 kinase and AP-1 DNA binding in cultured brain neurons: roles in glutamate excitotoxicity and lithium neuroprotection, J. Neurochem. 84: 566–575.
[368] O.J. Han, K.H. Joe. 2001. Involvement of p38 mitogen-activated protein kinase and apoptosis signal-regulating kinase-1 in nitric oxide-induced cell death in PC12 cells, Neurochem. Res. 26: 525–532.
[369] H. Kawasaki, et al. 1997. Activation and involvement of p38 mitogen activated protein kinase in glutamate-induced apoptosis in rat cerebellar granule cells, J. Biol. Chem. 272: 18518–18521.
[370] Y.J. Lee, H.N. Cho, et al. 2003. Oxidative stress-induced apoptosis is mediated by ERK1/2 phosphorylation, Exp. Cell Res. 291: 251–266.
[371] Fernyhough P., Gallagher A., et al. 1999. Abberant neurofilament phosphorylation in sensory neurons of rats with diabetic neuropathy. Diabetes **48:** 881–889.

[372] Fernyhough P. and Schmidt R. E. 2002. Neurofilaments in diabetic neuropathy. Int. Rev. Neurobiol. **50:** 115–144.
[373] Matthews C. C. and Feldman E. L. 1996. Insulin-like growth factor 1 rescues SH-SY5Y human neuroblastoma cells from hyperosmotic induced programmed cell death. J. Cell Physiol. **166:** 323–331.
[374] Russel J. W., Windebank A. J., et al. 1998. Insulin-like growth factor-1 prevents apoptosis in neurons after nerve growth factor withdrawal. J. Neurobiol. **36:** 455–467.
[375] Singleton J. R., Dixit V. M. and Feldman E. L. 1996. Type 1 insulin-like growth factor receptor activation regulates apoptotic proteins. J. Biol. Chem. **271:** 31791–31794.
[376] Cheng H. L. and Feldman E. L. 1998. Bi-directional regulation of p38 kinase and c-Jun N-terminal protein kinase by insulin-like growth factor-1. J. Biol. Chem. **273:** 14560–14565.
[377] Heck S., Lezonalc'h F., et al. 1999. Insulin like growth factor-1-mediated neuroprotection against oxidative stress is associated with nuclear factor kappa B. J. Biol. Chem. **274:** 9828–9835
[378] O'Reilly, D.D., Loomis, C.W., 2006. Increased expression of cyclooxygenase and nitric oxide isoform, and exaggerated sensitivity to prostaglandin E2, in the rat lumbar spinal cord 3 days after L5–L6 spinal nerve ligation. Anesthesiology 104: 328–337.
[379] Schmeichel, A.M., J.D. SCHMETZER. 2003. Oxidative injury and apoptosis of dorsal root ganglion neurons in chronic experimental diabetic neuropathy. Diabetes **52:** 165–171.
[380] Sima A. A. F. and Sugimoto K. 1999. Experimental diabetic neuropathy: an update. Diabetologia **42:** 773–788
[381] Sima A. A. F. 2001. Diabetic neuropathy; pathogenetic backgrounds, current and future therapies. Expert. Rev. Neurother. **1:** 225–238
[382] Tomlinson D. R. and Fernyhough P. 1999 Neurotrophism in diabetic neuropathy. In: Chronic Complications in Diabetes: Animal Models and Chronic Complications, pp. 167–182, Sima A.A.F. (ed.), Harwood, Amsterdam
[383] Li Z.-G., Zhang W. and Sima A. F. 2002. C-peptide prevents hippocampal apoptosis in type 1 diabetes. Int. J. Exp. Diabetes Res. **3:** 241–246.
[384] Sima A. A. F., Zhang W.-X., et al. 2001. C-peptide prevents and improves chronic type 1 diabetic neuropathy in the BB/Wor-rat. Diabetologia **44:** 889–897.
[385] Odergren T., Remahl S., and Wahren J. 1996. C-peptide improves autonomic nerve function in patients with type 1 diabetes. Diabetologia **39:** 687–695.
[386] Zhang W., Yorek M., Pierson C.R., et al. 2001. Human C-peptide dose dependently prevents early neuropathy in the BB/Wor-rat. Int. J. Exp. Diabetes Res. **2(3):** 187–194.
[387] Johansson B.-L., et al. 1999. Muscle vasodilatation by C-peptide is NO-mediated. Diabetologia **42:** A324.
[388] Ebendal T. 1992. Function and evolution in the NGF family and its receptors. J. Neurosci. Res. **32:** 461–470.
[389] Jakobsen J., Brimijoin S., et al. 1981. Retrograde axonal transport of transmitter enzymes, fucose-labeled protein, and nerve growth factor in streptozotocin- diabetic rats. Diabetes **30:** 797–803.

[390] Hellweg R. and Hartung H. D. 1990. Endogenous levels of nerve growth factor (NGF) are altered in experimental diabetes mellitus: a possible role for NGF in the pathogenesis of diabetic neuropathy. J. Neurosci. Res. **26:** 258–267.
[391] Brewster W. J., Fernyhough P., et al. 1994. Diabetic neuropathy, nerve growth factor and other neurotrophic factors. Trends Neurosci **17:** 321–325.
[392] Rueff A, et al. 1996. Characteristics of nerve growth factor induced hyperalgesia in adult rats: dependence on enhanced bradykinin-1 receptor activity but not neurokinin-1 receptor activity. Pain 66: 359–372.
[393] Bevan S, Winter J. 1995. Nerve growth factor (NGF) differentially regulates the chemosensitivity of adult rat cultured sensory neurons. J Neurosci 15: 4918–4926.
[394] Donnerer J, Schuligoi R, Stein C. 1992. Increased content and transport of substance P and calcitonin gene-related peptide in sensory nerves innervating inflamed tissue: evidence for a regulatory function of nerve growth factor in vivo. Neuroscience 49: 693–698.
[395] Malcangio M, Garrett NE, Tomlinson DR. 1997. Nerve growth factor treatment increases stimulus evoked release of sensory neuropeptides in the rat spinal cord. Eur J Neurosci 9: 1101–1104.
[396] Aruoma O. I., et al. 1988. The antioxidant action of taurine, hypotaurine and their metabolic precursors. Biochem. J. **256:** 251–255.
[397] El Idrissi A. and Trenkner E. 1999. Growth factors and taurine protect against excitotoxicity by stabilizing calcium homeostasis and energy metabolism. J. Neurosci. **19:** 9459–9468.
[398] Stevens M. J., Lattimer S. A., et al. 1993. Osmotically-induced nerve taurine depletion and the compatible osmolyte hypothesis in experimental diabetic neuropathy in the rat. Diabetologia **36:** 608–614.
[399] D. Kapur. 2003. Neuropathic pain and diabetes. Diabetes Met. Res. Rev.19: S9–S15.
[400] H. Shuangsong, T.J., et al. 2004. Early diabetic neuropathy is associated with differential changes in tetrodotoxin-sensitive and -resistant sodium channels in dorsal root ganglion neurons in the rat. J. Biol. Chem.
[401] C. Courteix, et al. 1993. Streptozocin-induced diabetic rats: behavioural evidence for a model of chronic pain, Pain 53: 81–88.
[402] S.H. Kim, J.M. Chung. 1992. An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat, Pain 50: 355–363.
[403] N.A. Calcutt,, et al. 1996. Tactile allodynia and formalin hyperalgesia in streptozotocin-diabetic rats: effects of insulin, aldose reductase inhibition and lidocaine, Pain 68: 293–299.
[404] B.S. Galer, A. Gianas, M.P. Jensen. 2000. Painful diabetic polyneuropathy: epidemiology, pain description, and quality of life, Diabetes Res. Clin. Pract. 47: 123–128.
[405] S.T. Krishnan, G. Rayman. 2003. New treatments for diabetic neuropathy: symptomatic treatments, Curr. Diab. Rep. 6: 3459–3467.
[406] C.P. Watson, D. Moulin, et al. 2003. Controlled-release oxycodone relieves neuropathic pain: a randomized controlled trial in painful diabetic neuropathy, Pain 105: 71–78.

[407] B.V. MacFarlane, et al. 1997. Chronic neuropathic pain and its control by drugs, Pharmacol. Ther. 75: 1–19.
[408] S.R. Chen, H.L. Pan. 2002. Hypersensitivity of spinothalamic tract neurons associated with diabetic neuropathic pain in rats, J. Neurophysiol. 82: 2726–2733.
[409] S.R. Chen, H.L. Pan. 2003. Antinociceptive effect of morphine, but not mu opioid receptor number, is attenuated in the spinal cord of diabetic rats, Anesthesiology 99: 1409–1414.
[410] M. Ohsawa, et al. 2000. Effects of a mu-opioid receptor agonist on G-protein activation in streptozotocin-induced diabetic mice, Eur. J. Pharmacol. 401: 55–58.
[411] M. Ohsawa, et al. 1998. Role of intracellular calcium in modification of mu and delta opioid receptor-mediated antinociception by diabetes in mice, J. Pharmacol. Exp. Ther. 286: 780–787.
[412] Manning, D. 2006. The role of neuroimmune activation in chronic neuropathic pain and new targets for therapeutic intervention. In Emerging Strategies for the Treatment of Neuropathic. In: Basbaum, AI.; Campbell, JN.; Dray, A.; Dubner, R.; Dworkin, RH.; Sang, CN., editors. Pain. Seattle, WA: IASP Press p. 161.-192.
[413] Wagner R, Myers RR. 1996. Endoneurial injection of TNF-alpha produces neuropathic pain behaviors. Neuroreport 7:2897–2901.
[414] Sorkin LS, Xiao WH, et al. 1997. Tumour necrosis factor-α induces ectopic activity in nociceptive primary afferent fibres. Neuroscience 81:255–262.
[415] Schäfers M, Lee DH, et al. 2003. Increased sensitivity of injured and adjacent uninjured rat primary sensory neurons to exogenous tumor necrosis factor-alpha after spinal nerve ligation. J Neurosci 23:3028–3038.
[416] Kanaan SA, Poole S, et al. 1998. Interleukin-10 reduces the endotoxin induced hyperalgesia in mice. J Neuroimmunol 86:142–150.
[417] Sugiura S, Lahav R, et al. 2006. Leukaemia inhibitory factor is required for normal inflammatory responses to injury in the peripheral and central nervous systems in vivo and is chemotactic for macrophages in vitro. Eur J Neurosci 12:457–466.
[418] Tofaris GK, Patterson PH, et al. 2002. Denervated Schwann cells attract macrophages by secretion of leukemia inhibitory factor (LIF) and monocyte chemoattractant protein-1 in a process regulated by interleukin-6 and LIF. J Neurosci 22:6696–6703.
[419] Abbadie C, Lindia JA, et al. 2003. Impaired neuropathic pain responses in mice lacking the chemokine receptor CCR2. Proc Natl Acad Sci USA 100:7947–7952.
[420] H.S. Huang, Q.H. Zhang, et al. 2002. Identification of gene expression profile of dorsal root ganglion in the rat peripheral axotomy model of neuropathic pain, Proc. Natl. Acad. Sci. U.S.A. 99: 8360–8365.
[421] L.J. Hudson, et al. 2001. VR1 protein expression increases in undamaged DRG neurons after partial nerve injury, Eur. J. Neurosci. 13: 2105–2114.
[422] Goya, P., Jagerovic, N., et al. 2003. Cannabinoids and neuropathic pain. Mini Rev. Med. Chem. 3: 765-772.
[423] Herzberg, U., Eliav, E., et al. 1997. The analgesic effects of R(C)-WIN 55,212-2 mesylate, a high affinity cannabinoid agonist, in a rat model of neuropathic pain. Neurosci. Lett. 221: 157-160.

[424] Bridges, D., et al. 2001b. The synthetic cannabinoid WIN55,212-2 attenuates hyperalgesia and allodynia in a rat model of neuropathic pain. Br. J. Pharmacol. 133: 586-594.
[425] Malan Jr., T.P., Ibrahim, M.M., et al. 2003. CB2 cannabinoid receptor agonists: pain relief without psychoactive effects? Curr. Opin. Pharmacol. 3: 62-67.
[426] Malan Jr., T.P., Ibrahim, M.M., et al. 2002. Inhibition of pain responses by activation of CB(2) cannabinoid receptors. Chem. Phys. Lipids 121: 191-200.
[427] Ibrahim, M.M., Deng, H., et al. 2003. Activation of CB2 cannabinoid receptors by AM1241 inhibits experimental neuropathic pain: pain inhibition by receptors not present in the CNS. Proc. Natl Acad. Sci. U.S.A. 100: 10529-10533.
[428] Costa, B., Colleoni, M., et al. 2004. Repeated treatment with the synthetic cannabinoid WIN 55,212-2 reduces both hyperalgesia and production of pronociceptive mediators in a rat model of neuropathic pain. Br. J. Pharmacol. 141: 4-8.
[429] Scott, D.A., Wright, C.E., Angus, J.A., 2004. Evidence that CB-1 and CB-2 cannabinoid receptors mediate antinociception in neuropathic pain in the rat. Pain 109: 124-131.
[430] W.J. Dixon. 1980. Efficiency analysis of experimental observations, Ann. Rev. Pharmacol. Toxicol. 20: 441–462.
[431] A. Dogrul, H. Gul, A., et al. 2003. Topical cannabinoid antinociception: synergy with spinal sites, Pain105: 11–16.
[432] J.A. Fuentes, M. Ruiz-Gayo, J., et al. 1999. Cannabinoids as potential new analgesics, Life Sciences 65: 675–685.
[433] M. Malcangio, D.R. Tomlinson. 1998. A pharmacological analysis of mechanical hyperalgesia in streptozocin/diabetic rats, Pain 76: 151–157.
[434] A. Fox, A. Kesingland, C., et al. 2001. The role of central and peripheral Cannabinoid1 receptors in the antihyperalgesic activity of cannabinoids in a model of neuropathic pain, Pain 92: 91–100.
[435] Z. Rudich, J. Stinson, et al. 2003. Treatment of chronic intractable neuropathic pain with dronabinol: case report of two adolescents, Pain Res. Manage. 8: 221–224.
[436] Rowbotham, M.C., 1995. Chronic pain: from theory to practical management. Neurology 45: S5-S10.
[437] Ahlgren SC, Levine JD. 1993. Mechanical hyperalgesia in streptozotocin-diabetic rats. Neuroscience 52: 1049–1055.
[438] Abrams, D. I., et al. 2007. Cannabis in painful HIV-associated sensory neuropathy: A randomized, placebo controlled trial. Neurology 68: 515-521.
[439] Nilsson G, Forsberg-Nilsson K, et al. 1997. Human mast cells express functional trkA and are a source of nerve growth factor. Eur J Immunol 27: 2295–2301.
[440] Lewin GR, Mendell LM. 1993. Nerve growth factor and nociception. Trends Neurosci 16: 353–359.
[441] Lewin GR, Rueff A, Mendell LM. 1994. Peripheral and central mechanisms of NGF-induced hyperalgesia. Eur J Neurosci 6: 1903–1912.
[442] Tal M, Liberman R. 1997. Local injection of nerve growth factor (NGF) triggers degrannulation of mast cells in rat paw. Neurosci Lett 221: 129–132.
[443] Leon A, Buriani A, et al. 1994. Mast cells synthesize, store and release nerve growth factor. Proc Natl Acad Sci USA 91: 3739–3743.

[444] Levi-Montalcini R, et al. 1996. Nerve growth factor: from neurotrophin to neurokine. Trends Neuroscience 19: 514–520.
[445] Friedel RH, et al. 1997. Identification of genes differentially expressed by nerve growth factor and neurotrophin-3 dependent sensory neurones. Proc Natl Acad Sci USA 94: 12670–12675.
[446] Hohmann AG, Herkenham M. 1999a. Localisation of central cannabinoid CB1 receptor messenger RNA in neuronal subpopulations of rat dorsal root ganglia: a double-label in situ hybridisation study. Neuroscience 90:923–931.
[447] Facci L, et al. 1995. Mast cells express a peripheral cannabinoid receptor with differential sensitivity to anandamide and palmitoylethanolamide. Proc Natl Acad Sci USA 92: 3376–3380.
[448] Melck D, De Petrocellis, L. et al. 1999. Suppression of nerve growth factor trk receptors and prolactin receptors by endocannabinoids leads to inhibition of human breast and prostate cancer cell proliferation. Endocrinology 141:118–126.
[449] Farquhar-Smith, W. P., et al. 2002. Attenuation of nerve growth factor-induced visceral hyperalgesia via cannabinoid CB1 and CB2-like receptors. Pain 97: 11-21.
[450] Hohmann AG, Herkenham M. 1999b. Cannabinoid receptors undergo axonal flow in sensory nerves. Neuroscience 92: 1171–1175.
[451] Piomelli, D., Giuffrida, A., et al. 2000. The endocannabinoid system as a target for therapeuticdrugs. Trends Pharmacol. Sci. 21: 218-224.
[452] Stander, S., Schmelz, M., et al. 2005. Distribution of cannabinoid receptor 1 (CB1) and 2 (CB2) on sensory nerve fibers and adnexal structures in human skin. J. Dermatol. Sci. 38: 177-188.
[453] Siegling, A., et al. 2001. Cannabinoid CB(1) receptor upregulation in a rat model of chronic neuropathic pain. Eur. J. Pharmacol. 415: R5-R7.
[454] Lim, G., et al. 2003. Upregulation of spinal cannabinoid-1-receptors following nerve injury enhances the effects of win55,212-2 on neuropathic pain behaviours in rats. Pain 105: 275-283.
[455] Zhang, J., Hoffert, C., et al. 2003. Induction of CB2 receptor expression in the rat spinal cord of neuropathic but not inflammatory chronic pain models. Eur. J. Neurosci. 17: 2750-2754.
[456] Spina, E., Perugi, G. 2004. Antiepileptic drugs: indications other than epilepsy. Epileptic Disord. 6: 57-75.
[457] Maizels, M., McCarberg, B., 2005. Antidepressants and antiepileptic drugs for chronic non-cancer pain. Am. Fam. Physician 71: 483-490.
[458] McQuay, H.J., Tramer, M., et al. 1996. A systematic review of antidepressants in neuropathic pain. Pain 68: 217-227.
[459] Sindrup, S.H., Jensen, T.S., 1999. Efficacy of pharmacological treatments of neuropathic pain: an update and effect related to mechanism of drug action. Pain 83: 389-400.
[460] Ban˜ os, J.E., Sa´ nchez, G., Berrendero, F., Maldonado, R., 2003. Neuropathic pain: some clues for future drug treatment. Mini Rev. Med. Chem. 3: 723-731.
[461] Foley, K.M., 2003. Opioids and chronic neuropathic pain. N. Engl. J. Med. 348: 1279-1281.

[462] Gee, N.S., Brown, J.P.,et al. 1996. The novel anticonvulsant drug, Gabapentin (Neurontin), binds to the alpha2delta subunit of a calcium channel. J. Biol. Chem. 271: 5768-5776.
[463] Rogawski, M.A., Loscher, W. 2004. The neurobiology of antiepileptic drugs. Nat. Rev. Neurosci. 5: 553-564.
[464] Mackie, K., Hille, B. 1992. Cannabinoids inhibit N-type Ca2C channels in neuroblastoma-glioma cells. Proc. Natl. Acad. Sci. USA 89, 3825–3829.
[465] Mackie, K., et al. 1995. Cannabinoids activate an inwardly-rectifying potassium conductance and inhibit Q-type voltage-dependent calcium currents. J. Neurosci. 15: 6552–6561.
[466] Pan, X., Ikeda, S.R., Lewis, D.L., 1996. Rat brain cannabinoid receptors modulates N type Ca2C channels in a neuronal expression system. Mol. Pharmacol. 49, 707–714.
[467] Twitchell, W., Brown, S., Maclie, K., 1997. Cannabinoid inhibits N- and P/Q-type calcium channels in cultured rat hippoacampal neurons. J. Neurophysiol. 78: 43–50.
[468] Iuvone, T., Esposito, G., et al. 2004. Neuroprotective effect of cannabidiol, a non-psychoactive component from Cannabis sativa, on beta-amyloid-induced toxicity in PC12 cells. J. Neurochem. 89: 134-141.
[469] Nagayama T, Sinor AD, et al. 1999. Cannabinoids and neuroprotection in global and focal cerebral ischemia and in neuronal cultures. *J Neurosci* **19:**2987–2995.
[470] Panikashvili D, Mechoulam R, et al. 2005. CB1 cannabinoid receptors are involved in neuroprotection via NF-kappa B inhibition. *J Cereb Blood Flow Metab* **25:**477–484.
[471] Parmentier-Batteur S, Jin K, et al. 2002. Increased severity of stroke in CB1 cannabinoid receptor knock-out mice. *J Neurosci* **22:** 9771–9775.
[472] Kim, S.H., et al. 2006. Molecular mechanisms of cannabinoid protection from neuronal excitotoxicity. Molecular Pharmacology 69: 691-696.
[473] M.H. Francisco, E. Pinteaux, L., et al. 2005. Neuroprotective effects of the synthetic cannabinoid HU-210 in primary cortical neurons are mediated by phosphatidylinositol 3-kinase/AKT signaling, Mol. Cell Neurosci. 28: 189–194.
[474] Chen, J., et al. 2005. Reactive oxygen species and p38 phosphorylation regulate the protective effect of delta-9 tetrahydrocannabinol in the apoptotic response to NMDA. Neuroscience Letters 389: 99-103.
[475] Du, X.L., et al. 2000. Hyperglycemia-induced mitochondrial superoxide overproduction activates the hexosamine pathway and induces PAI-1 expression by increasing Sp1 glycosylation. *Proc. Natl. Acad. Sci. USA*. **97**:12222–12226.
[476] M.B. Brownlee. 2002. Mechanism of hyperglycemic damage in diabetes, in: J.S. Skyler (Ed.), Atlas of Diabetes, second ed., Lippincott, Williams and Wilkins, Philadelphia, pp. 125–137.
[477] M. Morigi, S. Angioletti, et al. 1998. Leukocyte endothelial interaction is augmented by high glucose concentrations and hyperglycemia in a NF-kB-dependent fashion, J. Clin. Invest. 101: 1905–1915.
[478] N. Shanmugam, et al. 2003. High glucose-induced expression of proinflammatory cytokine and chemokine genes in monocytic cells, Diabetes 52: 1256–1264.
[479] Schaller B and Graf R. 2004. Cerebral ischemia and reperfusion: the pathophysiologic concept as a basis for clinical therapy. *J Cereb Blood Flow Metab* **24:**351–371.

[480] White BC, Sullivan JM, DeGracia DJ, O'Neil BJ, Neumar RW, Grossman LI, Rafols JA, and Krause GS. 2000. Brain ischemia and reperfusion: molecular mechanisms of neuronal injury. *J Neurol Sci* **179:** 1–33.

[481] Kim, S.H., et al. 2005. Involvement of protein kinase A in cannabinoid receptor mediated protection from oxidative neuronal injury. The Journal of Pharmacology and Experimental Therapeutics 313: 88-94.

[482] Boissel JP, Bros M, et al. 2004. Cyclic AMP-mediated upregulation of the expression of neuronal NO synthase in human A673 neuroepithelioma cells results in a decrease in the level of bioactive NO production: analysis of the signaling mechanisms that are involved. *Biochemistry* **43:**7197–7206.

[483] Yamagishi SI, et al. 2001. Leptin induces mitochondrial superoxide production and monocyte chemoattractant protein-1 expression in aortic endothelial cells by increasing fatty acid oxidation via protein kinase A. *J Biol Chem* **276:**25096–25100.

[484] Van Herreweghe F, Mao J, et al. 2002. Tumor necrosis factor-induced modulation of glyoxalase I activities through phosphorylation by PKA results in cell death and is accompanied by the formation of a specific methylglyoxal-derived AGE. *Proc Natl Acad Sci USA* **99:**949–954

[485] El Jamali A, et al. 2004. Reoxygenation after severe hypoxia induces cardiomyocyte hypertrophy in vitro: activation of CREB downstream of GSK3beta. *FASEB J* **18:**1096–1098.

[486] Childers SR and Deadwyler SA. 1996. Role of cyclic AMP in the actions of cannabinoid receptors. *Biochem Pharmacol* **52:**819–827.

[487] Jin KL, et al. 2000. CB1 cannabinoid receptor induction in experimental stroke. *Ann Neurol* **48:**257–261.

[488] Franklin A, Parmentier-Batteur S, et al. 2003. Palmitoylethanolamide increases after focal cerebral ischemia and potentiates microglial cell motility. *J Neurosci* **23:**7767–7775.

[489] Chen Y and Buck J. 2000. Cannabinoids protect cells from oxidative cell death: a receptor-independent mechanism. *J Pharmacol Exp Ther* **293:**807–812.

[490] S.K. Jain, et al. 1999. Effect of hyperketonemia on plasma lipid peroxidation levels in diabetic patients, Diabetes Care 22: 1171–1175.

[491] Costa, B., et al. 2007. The non-psychoactive cannabis constituent cannabidiol is an orally effective therapeutic agent in rat chronic inflammatory and neuropathic pain. European Journal of Pharmacology 556: 75-83.

[492] CONSROE, P., et al. 1991. Controlled clinical trial of cannabidiol in Huntington's disease. Pharmacol. Biochem. Behav. **40**(3)**:** 701–708.

[493] THOMPSON, J.A.,et al. . 1991. Relationship between the metabolism of butylated hydroxytoluene (BHT) and lung tumor promotion in mice Exp. Lung Res. **172**(2)**:** 439–453.

[494] LINDENSCHMIDT, R.C., et al. 1986. The effects of dietary butylated hydroxytoluene on liver and colon tumor development in mice. Toxicology **38**(2)**:** 151–160.

[495] Choi, D. W., Koh, J. Y.&Peters, S. 1988. *J. Neurosci.* **8,** 185–196.

[496] Hecker, M., Preib, C., Klemm, P., Busse, R., 1996. Inhibition by antioxidants of nitric oxide syntase expression in murine macrophages: role of nuclear factor κB and interferon regulatory factor 1. Br. J. Pharmacol. 118: 2178–2184.

[497] Subbaramaiah, K., Chung, W.J., Michaluart, P., Telang, N., Tanabe, T., Inoue, H., Jang, M., Pezzuto, J.M., Dannenberg, A.J., 1998. Resveratrol inhibits cyclooxygenase-2 transcription and activity in phorbol ester-treated human mammary epithelial cells. J. Biol. Chem. 273: 21875–21882.
[498] Turner, C.E., et al. 1981. Constituents of Cannabis sativa XVII: A review of the natural constituents. Jouranl of Natural products 43: 169-234.
[499] Asgary, S., et al. 1999. Anti-oxidant effect of flavanoids on hemoglobin glycosylation. Pharmacuetica actica Helvetiae 73: 223-226.
[500] Chabot, C., Massicote, M., Milot, F., Trudeau, J. & Gagne, J. 1997. Impaired modulation of AMPA receptors by calcium-dependent processes in streptozotocin-induced diabetic rats. Brain Res., 768, 249–256.
[501] Gardoni, F., Kamal, A., Bellone, C., Biessels, G.J., Ramakers, G., Cattabeni, F., Gispen, W.H. & Di Luca, M. 2002. Effects of streptozotocin-diabetes on the hippocampal NMDA receptor complex in rats. J. Neurochem., 80, 438–447.
[502] Valastro, B., Cossette, N., Lavoie, N., Gagnon, F., Trudeau, J. & Massicote, M. 2002. Up-regulation of glutamate receptors is associated with LTP defects in the early stages of diabetes mellitus. Diabetologia, 45, 642–650.
[503] Kamal, A., Biessels, G.J., Urban, I. & Gispen, W.H. 1999. Hippocampal synaptic plasticity in streptozotocin-diabetic rats: interaction of diabetes and ageing. Neuroscience, 90, 737–745.
[504] Magarin˜ os, A. & McEwen, B. 2000. Experimental diabetes in rats causes hippocampal dendritic and synaptic reorganization and increased glucocorticoid reactivity to stress. Proc. Natl Acad. Sci. U.S.A., 97, 11 056–11 061.
[505] Revsin, Y., Saravia, F., Roig, P., Lima, A., de Kloet, E.R., Homo-Delarche, F. & De Nicola, A.F. 2005. Neuronal and astroglial alterations in the hippocampus of a mouse model for type 1 diabetes. Brain Res., 1038, 22–31.
[506] Beauquis, J. 2006. Reduced hippocampal neurogenesis and number of hilar neurons in streptotozocin-induced diabetic mice: reversion by antidepressant treatment. European Journal of Neuroscience 23: 1539-1546.
[507] Lustman, P., Freedland, K., Griffith, L. & Clouse, R. 2000. Fluoxetine for depression in diabetes: a randomized double-blind placebo-controlled trial. Diabetes Care, 23, 618–623.
[508] Madsen, T., Treschow, A., Bengzon, J., Bolwig, T., Lindvall, O. & Tingtrom, A. 2000. Increased neurogenesis in a model of electroconvulsive therapy. Biol. Psychiat., 47: 1043–1049.
[509] Malberg, J., Eisch, A., Nestler, E. & Duman, R. 2000. Chronic antidepressant treatment increases neurogenesis in adult hippocampus. J. Neurosci., 20, 9104–9110.
[510] Malberg, J. 2004. Implications of adult hippocampal neurogenesis in antidepressant action. Rev. Psychiat. Neurosci., 29, 196–205.
[511] Czeh, B., Michaelis, T., et al. 2001. Stress-induced changes in cerebral metabolites, hippocampal volume, and cell proliferation are prevented by antidepressant treatment with tianeptine. Proc. Natl Acad. Sci. U.S.A., 98, 12 796–12 801.
[512] Duman, R., Malberg, J. & Nakagawa, S. 2001. Regulation of adult neurogenesis by psychotropic drugs and stress. J. Pharmacol. Exp. Therap., 299, 401–407.
[513] Jacobs, B. 2002. Adult brain neurogenesis and depression. Brain Behav. Immun., 16, 602–609.

[514] Santarelli, L., Saxe, M., Gross, C., et al. 2003. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science, 301, 805–809.
[515] Schnur, Matthew. 2006. Anxiety and Depression Petition.
[516] Sapolsky, R. 2004. Is impaired neurogenesis relevant to the affective symptoms of depression? Biol. Psychiat., 56, 137–139.
[517] Singer, D.E., Nathan, D.M., et al. 1992. Association of HbA1c with prevalence of cardiovascular disease in the original cohort of the Framingham Study. *Diabetes.* **41**: 202–208.

[518] Laakso, M. 1999. Hyperglycemia and cardiovascular disease in type 2 diabetes. *Diabetes*. **48**: 937–942.
[519] Jensen-Urstad, K.J., et al. 1996. Early atherosclerosis is retarded by improved long-term blood glucose control in patients with IDDM. *Diabetes.* **45**:1253–1258.
[520] Turner, R.C., et al. 1998. Risk factors for coronary artery disease in non insulin dependent diabetes mellitus: United Kingdom Prospective Diabetes Study (UKPDS:23). *BMJ.* **316**:823–828.
[521] Jeanrenaud B. 1994. Central nervous system and peripheral abnormalities: clues to the understanding of obesity and NIDDM. *Diabetologia* 37 (Suppl. 2):S170–S178.
[522] Koyama K, Chen G, Lee Y, Unger RH. 1997. Tissue triglycerides, insulin resistance, and insulin production: implications for hyperinsulinemia in obesity. *Am J Physiol* 273: E708–E713.
[523] Fletcher JM, McKenzie N. 1988. The parasympathetic nervous system and glucocorticoid- mediated hyperinsulinemia in the genetically obese (fa/fa) Zucker rat. *J Endocrinol* 118:87–92.
[524] Penicaud L, Rohner-Jeanrenaud F, Jeanrenaud B. 1986. In vivo metabolic changes as studied longitudinally after ventromedial hypothalamic lesions. *Am J Physiol* 250:E662–E668.
[525] Zhou YP, Cockburn BN, Pugh W, Polonsky KS. 1999. Basal insulin hypersecretion in insulin-resistant Zucker diabetic and Zucker fatty rats: role of enhanced fuel metabolism. *Metabolism* 48:857–864.
[526] Weyer C, Salbe AD, Lindsay R, Bogardus C, Pratley RE, Tataranni PA. Exaggerated pancreatic polypeptide secretion in Pima Indians: can increased parasympathetic drive to the pancreas contribute to hyperinsulinemia and diabetes in humans? *Metabolism.* In press.
[527] DeFronzo RA. 1997. Pathogenesis of type 2 diabetes: metabolic and molecular implications for identifying diabetes genes. *Diabetes Rev* 3:177–269.
[528] Warram JH, Martin BC, et al. 1990. Slow glucose removal rate and hyperinsulinemia precede the development of type 2 diabetes in the offspring of diabetic parents. *Ann Intern Med* 113:909–915.
[529] Martin BC, Warram JH, et al. 1992. Role of glucose and insulin resistance in development of type 2 diabetes: results of a 25-year follow-up study. *Lancet* 340: 925–929.
[530] Warram JH, et al. 1996. Natural history of impaired glucose tolerance: follow-up at Joslin Clinic. *Diabet Med* 13:S40–S45.
[531] Charles MA, et al. 1991. Risk factors for NIDDM in white population: Paris prospective study. *Diabetes* 40:796–799.

[532] Lundgren H, 1990. Fasting serum insulin concentration and early phase insulin response as risk determinants for developing diabetes. *Diabet Med* 7:407–413.
[533] Erriksson KF, Lindgarde F. 1996. Poor physical fitness, and impaired early phase insulin response but late hyperinsulinemia as predictors of NIDDM in middle aged Swedish men. *Diabetologia* 39:573–579.
[534] Skarfors E, Selinus K, Lithell H. 1991. Risk factors for developing non-insulin dependent diabetes: a 10-year follow up of men in Uppsala. *BMJ* 303:755–760.
[535] Bergstrom RW, Newell-Morris LL, et al. 1990. Association of elevated fasting C-peptide level and increased intraabdominal fat distribution with development of NIDDM in Japanese-American men. *Diabetes* 39:104–111.
[536] Miles PDG, Li S, et al. 1998. Mechanisms of insulin resistance in experimental hyperinsulinemic dogs. *J Clin Invest* 101: 202–211.
[537] Leahy JL. 1990. Natural history of _-cell dysfunction in NIDDM. *Diabetes Care* 13: 992-1010.
[538] Reaven GM: Banting Lecture. 1988. Role of insulin resistance in human disease. *Diabetes* 37:1595–1607.
[539] DeFronzo RA: Lilly Lecture 1988. The triumvirate: B-cell, muscle, liver: a collision responsible for NIDDM. *Diabetes* 37:667–687.
[540] Pratley RE, Weyer C, Bogardus. 2000. Metabolic abnormalities in the development of non-insulin dependent diabetes mellitus. In *Diabetes Mellitus.* 2nd ed. LeRoith D, Taylor SI, Olefsky JM, Eds. Philadelphia, Lippincot-Raven p. 548–557.
[541] C.K. Buffington, et al. 1986. Phytohemagglutinin (PHA) activated human T-lymphocytes: insulin binding in T-lymphocytes, Biochem. Biophys. Res. Commun. 134: 412–419.
[542] J.H. Helderman. 1981. Role of insulin in the intermediary metabolism of the activated thymic-derived lymphocyte, J. Clin. Invest 67: 636–1642.
[543] L. Ercolani, H.L. Lin, B.H. Ginsberg. 1985. Insulin-induced desensitization at the receptor and post-receptor level in mitogen-activated T-lymphocytes, Diabetes 34: 931–937.
[544] T.J. Brown, L. Ercolani, B.H. Ginsberg. 1983. Properties and regulation of the T-lymphocyte receptor, J. Recept. Res. 3: 481–494.
[545] F.B. Stentz, A.E. Kitabchi. 2003. Activated T-lymphocytes in type 2 diabetes: implications for in vitro studies, Curr. Drug Targets 4: 493–503.
[546] F.B. Stentz, A.E. Kitabchi. 2004. De novo emergence of growth factor receptors in activated human CD4+ and CD8+ T-lymphocytes, Metabolism 53: 117–122.
[547] F.B. Stentz, et al. 2004. Proinflammatory cytokines, markers of cardiovascular risks, oxidative stress and lipid peroxidation in patients with hyperglycemic crisis, Diabetes 53: 2079–2086.

[548] M. Prentki, F.M. Matschinsky. 1987. Ca2+, cAMP, and phospholipidderived messengers in coupling mechanisms of insulin secretion, Physiol. Rev. 67: 1185–1248.
[549] F.M. Ashcroft, P. Rorsman. 1989. Electrophysiology of the pancreatic beta cell, Prog. Biophys. Mol. Biol. 54: 87–143.
[550] R.M. Santos, et al. 1991. Widespread synchronous [Ca2+]i oscillations due to bursting electrical activity in single pancreatic islets, Pflugers Arch. 418: 417–422.

[551] Kreitzer, A.C., et al. 2001. Retrograde inhibition of presynaptic calcium influx by endogenous cannabinoids at excitatory synapses onto Purkinje cells. Neuron 29: 717-727.
[552] Caulfield MP, Brown DA. 1992. Cannabinoid receptor agonists inhibit Ca current in NG108-15 neuroblastoma cells via a pertussis toxin-sensitive mechanism. Br J Pharmacol 106: 231-2.
[553] Wilson RI, Nicoll RA. 2001. Endogenous cannabinoids mediate retrograde signaling at hippocampal synapses. Nature 410: 588-592
[554] Juan-Pico, Pablo, et al. 2006. Cannabinoid receptors regulate Ca2+ signals and insulin secretion in pancreatic β-cell. Cell Calcium 39: 155-162.
[555] Gasperi, V., et al. 2006. Endocannabinoids in adipocytes during differentiation and their role in glucose uptake. Cell Mol. Life Sci. 18: 5445.
[556] Hillard, C. J., Manna, S., et al. 1999. Synthesis and characterization of potent and selective agonists of the neuronal cannabinoid receptor (CB1). J. Pharmacol. Exp. Ther. 289: 1427–1433.
[557] Roy, D., Perreault, M. and Marette, A. 1998. Insulin stimulation of glucose uptake in skeletal muscles and adipose tissues in vivo is NO dependent. Am. J. Physiol. 274: 692–6
[558] Maccarrone, M., et al. 2000. Anandamide uptake by human endothelial cells and its regulation by nitric oxide. J. Biol. Chem. 275: 13484–13492.
[559] Nugent, C., et al. 2001. Arachidonic acid stimulates glucose uptake in 3T3-L1 adipocytes by increasing GLUT1 and GLUT4 levels at the plasma membrane. Evidence for involvement of lipoxygenase metabolites and peroxisome proliferator-activated receptor gamma. J. Biol. Chem. 276: 9149–9157.
[560] Chatzipanteli, K., Rudolph, S. and Axelrod, L. 1992. Coordinate control of lipolysis by prostaglandin E2 and prostacyclin in rat adipose tissue. Diabetes 41: 927–935.
[561] Girouard, H. and Savard, R. 1998. The lack of bimodality in the effects of endogenous and exogenous prostaglandins on fat cell lipolysis in rats. Prostaglandins Other Lipid Mediat. 56: 43–52.
[562] Gomez del Pulgar T, et al. 2000. The CB1 cannabinoid receptor is coupled to the activation of protein kinase B/Akt. *Biochem J* **347:** 369–373.
[563] Molina-Holgado, Francisco, et al. 2005. Neuroprotective effects of the synthetic cannabinoid HU-210 in primary cortical neurons are mediated by phosphoinositol-3 kinase/AKT signaling. Molecular and Cellular Neuroscience 28: 189-194.
[564] Bouaboula M., Perrachon S., Milligan L. et al. 1997. A selective inverse agonist for central cannabinoid receptor inhibits mitogen activated protein kinase activation stimulated by insulin or insulin-like growth factor 1 - Evidence for a new model of receptor/ligand interactions. *J Biol Chem* **272**: 22330–22339.
[565] McAllister, S.D., and Michelle Glass. 2002. CB1 and CB2 receptor mediated signaling: A focus on endocannabinoids. Prostaglandins, Leukotrienes, and Essential Fatty Acids 66: 161-171.
[566] Bouaboula M., Poinotchazel C., Bourrie B. et al. 1995. Activation of mitogen-activated protein–kianase by stimulation of the central cannabinoid receptor CB1. *Biochem J* **312**: 637–641.

[567] Bouaboula M., Poinotchazel C., Marchand J. et al. 1996. Signaling pathway associated with stimulation of CB2 peripheral cannabinoid receptor - Involvement of both mitogen-activated protein kinase and induction of Krox-24 expression. *Eur J Biochem* **237**: 704–711.
[568] Wartmann M., Campbell D., et al. 1995. The MAP kinase signal transduction pathway is activated by the endogenous cannabinoid anandamide. *FEBS Lett* **359**: 133–136.