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Effects of Insulin and Oral Antidiabetic Agents on Glucose Metabolism, Vascular Dysfunction and Skeletal Muscle Inflammation in Type 2 Diabetic Subjects*

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Abstract

Background—To test potential differences between the actions of anti-diabetic medications, we examined the effects of oral hypoglycemic agents versus insulin glargine-apidra therapy in T2DM.

Methods—T2DM subjects were randomized to either OHA (pioglitazone, metformin, glipizide, n=9) or IT (n=12) for 6 months. Carotid intimal media thickness (CIMT), vascular reactivity [FMD] (% change in brachial arterial diameter [BD] post-ischemia), and sublingual nitrate [SLN] were measured with ultrasonography. Euglycemic hyperinsulinemic (80 mU/m²) clamp with [3]-³H-glucose and muscle biopsies were performed.

Results—FPG (~257 to ~124, OHA ~256 to ~142 mg/dl, IT) and HgbA_{1C} (~10.3 to ~6.4%, OHA; ~10.7 to ~7.1%, IT), improved comparably. EGP (~2.1 to ~1.7, OHA; ~2.3 to ~2.0, IT) and EGP suppression by insulin (~0.4 to ~0.3, OHA; ~0.5 to ~0.7 mg/kg.min, IT) was different. Total glucose disposal [TGD/I] × 100 increased in OHA (~5.2 to ~8.1, p=0.03), but not in IT (~6.0 to ~5.4 mg/kg.min/μU/ml × 100). OHA reduced CIMT (~0.080 to ~0.068, p<0.05), whereas IT did not (~0.075 to ~0.072 cm). After SLN, BD increased in OHA (~8.7 to ~18.2%), but not in IT (~11.2 to ~15.0), [p<0.02]. Except for plasma adiponectin (~7 to ~15, OHA vs. ~6 to ~10, IT), changes in inflammatory markers in the circulation and in muscle (IkBα, SOD2 & MCP1, p-ERK and JNK) were equivalent.

Conclusions—OHA and IT achieved adequate glycemic control and the effects on circulating and muscle inflammatory biomarkers were similar, but only OHA improved insulin sensitivity, vascular function and CIMT. These findings in a small sample suggest that the use of OHA provide additional benefits to patients with T2DM.

Keywords

Basal-Bolus Therapy; Oral Agents; Vascular Dysfunction; Muscle Tissue Inflammatory Markers; Glycemic Control

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Introduction

Atherosclerotic cardiovascular disease (ASCVD) represents the major cause of death in patients with type 2 diabetes [T2DM]. Insulin resistance, a characteristic feature of T2DM, particularly in Mexican Americans, is accompanied by an increase in cardiovascular (CV) morbidity and mortality (1). Current concepts indicate that atherosclerosis begins early in the natural history in T2DM and is associated with vascular inflammation in the arterial wall. Because the ultra-structure of the vessel cannot be accessed with biopsies during clinical studies in humans, cellular and molecular findings in skeletal muscle (2) and adipose tissue (3) have been used as surrogate markers of the inflammatory process in the arterial wall. Likewise, carotid intimal media thickness (CIMT) and the arterial response to ischemia and vasoactive agents have been used as evidence of large vessels atherogenesis and as measures of vascular endothelial function, respectively (4). Although improved glycemic control has been shown to decrease CV events in T2DM (5), large trials using insulin therapy have failed to demonstrate a beneficial effect to reduce CV events (6, 7). Considerable evidence suggests that insulin especially in high doses may be atherogenic (8). With respect to this, little is known about the effects of intensive insulin therapy on surrogate measures of ASCVD, i.e., CIMT, vascular endothelial function and pro-inflammatory cytokines (9). Therefore, a direct comparison between the effects of intensive insulin therapy vs. oral hypoglycemic agents is of considerable interest especially in Mexican American T2DM, in whom intensive insulin therapy has been poorly characterized.

In the present study, we compared the ability of glargine-glulisine combination intensive insulin therapy versus those of oral agents (Pioglitazone, Metformin and Sulfonylurea) to reach comparable glycemic and lipemic control and to improve insulin resistance, vascular endothelial dysfunction and CIMT. We examined the effects of both regimes on cytokines and key inflammatory pathways (IkB/NFκB, JNK and ERK) in skeletal muscle.

Methods

Experimental Design

Subjects—Twenty-one T2DM subjects of Mexican American descent with HbA_{1c} >7% were recruited from the outpatient clinic at the Texas Diabetes Institute (TDI). Table 1 summarizes the subject characteristics before and after 6 months of therapy. All had stable body weight ($\pm$ 3 lbs) for at least 3 months prior to the study and were not taking any medications known to affect glucose metabolism. No patient had evidence of renal, CV, or liver, as determined by the medical history, physical exam, blood tests, urinalysis and electrocardiogram. The protocol was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio and all subjects signed informed consent before participation in the study.

Experimental Protocol—Subjects randomly were assigned to receive therapy with oral hypoglycemic agents (OHA, n=9) or insulin (n=12). At entry, age, gender, BMI, % body fat, blood pressure, HgbA_{1c}, plasma lipids and diabetes duration were similar between the two groups. Medications use was comparable in both groups (Table 1). To achieve a fasting plasma glucose (FPG)<110mg/dl over a period of 4 weeks in the OHA, therapy was initiated

with pioglitazone, 15 mg/day titrated up to 45 mg/day in 6 weeks. Seven patients required the addition of metformin, started at 1000 mg/day and titrated up to 2000 mg/day, to reduce FPG <110 mg/dl. Four patients required additional glipizide, 10 mg/day to achieve target FPG. Twelve patients received insulin glargine (mean dose of 35 ± 12 Units/day) plus pre-meal glulisine (mean dose 28 ± 6 Units/day). The basal-bolus insulin was adjusted to reduce the FPG <110 mg/dl and the post-meal glucose <150 mg/dl. All subjects received nutritional advice about a weight-maintaining diet (45-50% CHO; 30-35% FAT and 20% protein) by a dietitian at the TDI and monitored capillary blood glucose four times daily. The goal was to adjust oral agents and the insulin dose to achieve comparable HbA_{1c} equal or below 7.0% as quickly as possible.

Euglycemic Insulin Clamp: Insulin resistance was quantitated with the euglycemic hyperinsulinemic clamp technique [80 mU/m².min]), in combination with tritiated glucose infusion (10). Briefly, patients reported to the Clinical Research Unit at 7:00 AM after a 10-12 hour overnight fast. Catheters were inserted into an antecubital vein (for all infusions) and retrograde hand vein (for blood sampling), which was placed in a heated box (65° C) to provide arterialized blood. At time t = -180 minutes, a primed ($25 \mu\text{Ci} \times \text{FPG}/100$)-continuous (0.25 $\mu\text{Ci}/\text{min}$) infusion of 3-[³H] glucose (Perkin Elmer, Boston, MA) was started. At time t = -120 minutes, a percutaneous biopsy of the vastus lateralis muscle was performed under local anesthesia, as previously reported (11). At time t = -30 min to 0 min baseline blood samples were obtained every 5-10 minutes for measurement of plasma glucose, insulin, free fatty acids (FFA) concentrations and tritiated glucose specific activity. At time = 0 min, insulin was infused at 80 mU/m².min for 180 minutes to raise the plasma insulin concentration to ~120 $\mu\text{U}/\text{ml}$. When the plasma glucose concentration declined to 100 mg/dl a variable infusion of 20% dextrose was appropriately adjusted to maintain the plasma glucose concentration constant at this level ($\pm 5\%$). Two additional muscle biopsies were performed during the insulin infusion period in opposite legs, at 30 minutes and again at 180 minutes. During the insulin clamp, a small amount of arterialized blood (0.5 ml) was withdrawn every 5 minutes to determine plasma glucose concentration. During the insulin clamp non-steady state conditions prevail and rates of glucose appearance and disappearance are calculated using Steele's equation (12).

Body Composition: Subjects returned to the Clinical Research Center one week after the euglycemic insulin clamp for body composition analysis and vascular studies after a 10-12 h overnight fast. Approximately 30 minutes before the vascular studies were started body composition was determined by DEXA (Hologic, Bedford, MA). The acquired images were integrated and analyzed by a software computer program and total amount of fat, fat-free mass and total body water were determined.

Vascular Studies and Inflammatory Biomarkers: Vascular dynamic tests (13,14) were performed 30 minutes after DEXA was completed. To assess vascular reactivity high-resolution ultrasonography (LOGIq-9, GE Chicago, IL) of the left brachial artery was performed at baseline, during reactive hyperemia (endothelial-mediated) and following administration of sublingual nitroglycerin. Prior to starting the studies patients remained in the supine position for 30 minutes in a quiet room with stable temperature. After the brachial

artery was identified, a 10 MHz external probe was applied and the probe was fixed to the skin. All images were acquired simultaneously with an electrocardiogram. Five baseline images were acquired to determine brachial arterial diameter and flow velocity. Then, a forearm pressure cuff was inflated to 250 mmHg for five minutes to create transient ischemia. Upon the release of the cuff the brachial artery diameter and flow velocity (reactive hyperemia) were measured continuously for 300 seconds. Ten minutes after completion of the reactive hyperemia study, restoration of baseline vascular parameters was ascertained and five repeat consecutive determinations of brachial arterial diameter and flow velocity were obtained. One tablet (0.4 mg) of sublingual nitroglycerin was applied and after 3 minutes the brachial arterial diameter and flow velocity were measured continuously for 2 minutes. After completion of this study, the right common carotid artery was identified and the external probe was placed on the overlying skin. Five consecutive images of the common carotid artery 1 cm proximal to the arterial bifurcation were acquired and stored for posterior analysis. The carotid IMT was determined as the average value for each individual and is presented in cm. All images were stored, and the data were analyzed independently by two observers on a separate day. Blood pressure and pulse rate were monitored throughout the entire duration of the vascular studies. Brachial arterial diameter was measured at 40-60 seconds after cuff release. The mean value for each test, endothelial-dependent (reactive hyperemia) and endothelial-independent (post-nitroglycerin), Flow-Mediated vasodilatation (FMD) was calculated, and these data are presented as percent change from baseline.

Blood samples were collected for determination of baseline circulating levels of leptin, adiponectin, adhesion molecules VCAM and ICAM, high sensitivity C-reactive protein (hsCRP), TNF- α , interleukin-6, endothelin-1 (ET-1), plasma lipid profile (total/HDL/LDL cholesterol and triglycerides concentration), LDL and HDL particle size and apolipoproteins A1, B and C-III concentration.

Skeletal Muscle Analysis (Western blotting): Muscle biopsy sample were debrided of fat and connective tissue and immediately frozen in liquid nitrogen. The tissue was homogenized as previous described (15). For Western blotting analysis, proteins (50 μ g) from the total muscle lysates were separated by 10% SDS-PAGE and transferred to a nitrocellulose membrane. After blocking the membranes with 5% BSA/TBST, they were probed overnight at 4°C with antibodies against human I κ B- α , p-JNK, JNK, p-ERK and ERK (all from Cell Signaling Technologies, Beverly, MA). Bound antibody was detected using anti-rabbit immunoglobulin-horseradish peroxidase-linked antibody and ECL reagents (Perkin Elmer, Boston, MA). Protein content of the active (phosphorylated) form of p-JNK1/2, and p-ERK1/2 are corrected to total JNK1/2 and ERK1/2 content and are expressed as a ratio.

RNA isolation, reverse transcription and real-time PCR: Total RNA was extracted from muscle tissue using Trizol Reagent (Invitrogen, Carlsbad, CA, USA) and purified. RNA concentration was measured by spectrophotometer using the Nanodrop 1000 (Thermo Scientific, Beverly, MA). Quantitative real Time PCR was performed with ABI 7500 rtPCR system (Applied Biosystem, Beverly, MA) using TaqMan One-Step RT-PCR Master Mix Assay on Demand primer/probes. (18s: Hs99999901-sl, monocyte-chemo-attractant protein

1 [MCP1]: Hs00234140_ml and super-oxidase dismutase 2 [SOD2]: Hs00167309_ml). RNA content was calculated from the cycle threshold values by using a standard curve constructed from a serial dilution of skeletal muscle total RNA standard (16). Results are compared between pre vs. post-therapy.

Randomization: After completion of all baseline studies, patients were randomized by the investigator to glargine-apidra basal-bolus subcutaneous insulin injections (IT, n= 12) or oral hypoglycemic agents (OHA, n=9). The initial dose of basal insulin glargine was estimated to represent 50% of the total daily dose, which was calculated as 0.25 units/kg ideal body weight. The remaining 50% of the total daily insulin was divided in three pre-meal boluses of insulin apidra. Some patients assigned to the IT group had sulfonylurea (n=4) discontinued. The insulin dose in the basal-bolus regimen (IT) and the pioglitazone, metformin and sulfonylurea (OHA) were titrated as described the *Follow-up* section.

Follow-up: All patients were instructed to measure capillary blood glucose frequently (4-6 times daily) and insulin adjustments (every week) or insulin supplementation (daily) was encouraged. Insulin glargine was given either in the morning (n=9), preferably, and before bedtime (n=3), according to patient's choice. The glargine dose was adjusted down or up by 10% if morning fasting capillary glucose was <80 mg/dl or >110 mg/dl, respectively. To achieve and maintain the 2 hr postprandial blood glucose <150 mg/dl, a daily supplemental scale with the addition of one, two or three units of insulin apidra before each meal was recommended, if the pre-prandial capillary blood glucose was >110, >150 or >180 mg/dl, respectively. All values were entered in a diary by the patient and reviewed by the physician/nurse weekly by phone and every 4 weeks during scheduled visits. Episodes of severe hypoglycemia and periods of sustained hyperglycemia above 200 mg/dl required a visit to the physician/nurse at the TDI for insulin adjustments. Therapy of patients in the OHA group was started with pioglitazone at 15 mg/day initially and increased to 30 mg/day after 4 weeks and to 45 mg/day in 6 weeks, as tolerated. In seven patients, metformin was added at 1000 mg twice daily and up to 2000 mg/day within two weeks as necessary to achieve the glycemic target. Four patients required the addition of glipizide 5 mg twice daily to reach fasting blood glucose <110 mg/dl. The goal of therapy was to achieve HbA_{1c} ≈7.0% in both groups, as quickly as possible (~ 2 months). All participants were contacted weekly by phone to ascertain that there were no serious adverse events during the initial 4 weeks. All subjects were required to return to the TDI at 4-week intervals during the entire 6-month study period. At each visit, history and a brief physical exam with routine laboratory values were performed. At 3 and 6 months, subjects had HgbA_{1c} chemistry panel, urine microalbumin/creatinine and fasting lipid profile measured. At the end of the 6-month follow up period, all studies (euglycemic insulin clamp, DEXA, vascular tests, CIMT and circulating inflammatory markers) were repeated.

Laboratory analyses: Fasting plasma glucose (FPG) was measured by the Beckman Glucose Analyzer (Beckman Instruments, Fullerton, CA) and HbA_{1c} by HPLC using DCA 2000+ analyzer (Bayer HealthCare LLC, Elkhart, IN) (17). Insulin was determined by radioimmunoassay (Diagnostic Products, Los Angeles, CA) (18) and free fatty acids (FFA) by colorimetric assay (Wako, MA) (19). Plasma concentration of VCAM, ICAM, TNF- α , hsCRP, ET-1 (R&D Systems, Minneapolis, MN) (20); leptin and adiponectin (LINCO

Research, Missouri) (21) were measured by ELISA assays and plasma lipidogram with apolipoprotein by MRS (Liposcience, Chapel Hill, NC) (22).

Statistical Analyses: The sample size calculation was based on data obtained on the FMD response to acetylcholine and sodium nitroprusside in normal and diabetic subjects in our preliminary studies and from ultrasonography data reported in the literature. The pooled standard deviation for changes from baseline to endpoint in forearm blood (BF) in our laboratory is 1.20 ml/100gr per min. In order to detect with 80% power a >20% difference in BF, and FMD in response to ischemia before and after 6 months of therapy, 9 completed patients in each arm were required. Data are expressed as mean $\pm$ SEM, except when indicated otherwise.

Changes from baseline to 6 months within each group were evaluated with the paired *T* test. Changes between groups were analyzed using *T* test for independent groups. The molecular results from muscle samples were compared using ANOVA with repeated measures over time. Associations between variables were determined with Pearson's correlation. Statistical significance was determined as a *p* 0.05.

Results

Subject characteristics at baseline and after 6 months of therapy with either hypoglycemic agents (OHA) or with combination glargine-apidra insulin therapy (IT) are summarized on Table 1. Patients randomized to insulin therapy had longer duration of the disease (*p*<0.05) and gained less body weight, with less absolute body fat mass (*p*<0.05) after 6 months of therapy. The reductions in FPG and HbA_{1c} were equivalent in both groups after 6 months, and blood pressure remained the same. There were 2 episodes of non-serious hypoglycemia in IT and none in OHA. The changes in plasma total, LDL and HDL-cholesterol and in plasma triglyceride concentrations, as well as the changes in lipid particles size were comparable between groups (Table 2). The increases in plasma adiponectin and leptin levels were greater in the OHA than in IT (*p*<0.001), but no significant changes were documented in the pro-inflammatory biomarkers measured in the circulation, including C-reactive protein, adhesion molecules, interleukin-6 and tumor necrosis factor alpha, and in plasma endothelin-1 (Table 2).

Glucose and Fatty Acid Metabolism (Table 3)

Prior to the initiation of therapy, basal EGP was similar and declined equally after 6 months in OHA (2.10 ± 0.20 to 1.77 ± 0.20 and IT 2.14 ± 0.26 to 1.97 ± 0.23 mg.kg⁻¹.min⁻¹). Insulin suppression during the clamp before and after therapy was also similar (0.42 ± 0.18 and 0.37 ± 0.16 in OHA, and 0.47 ± 0.12 and 0.66 ± 0.20 mg.kg⁻¹.min⁻¹, in IT). Both fasting plasma FFA concentration (393 ± 39 vs. 520 ± 40 mEq/L) and the suppression of plasma FFA during the insulin clamp (55 ± 15 vs. 110 ± 20 mEq/L) were significantly greater in the OHA vs. IT (*p*<0.01 and *p*<0.05, respectively) after 6 months of therapy. Fasting insulin concentration decreased with OHA and increased with IT, but the differences did not reach statistical significance. Total glucose disposal factored by the steady-state plasma insulin concentration and expressed as $[100 \times (\text{TGD}/\text{SSPI})]$ increased from 5.2 ± 0.5 to 8.1 ± 0.8 in

OHA and decreased from 6.0 ± 0.9 to 4.7 ± 0.5 $\text{mg/kg}^{-1} \cdot \text{min}^{-1}/\mu\text{U/ml}$ in IT after 6 months ($p < 0.05$ between groups).

Vascular Function Tests and CIMT Measurements (Table 4)

Progression of CIMT was attenuated by OHA therapy (0.080 ± 0.004 to 0.068 ± 0.003 cm), whereas IT had no effect (0.075 ± 0.004 to 0.072 ± 0.006 cm) ($p < 0.05$ between groups). The post-ischemic increase in brachial arterial diameter following OHA therapy (9.8 ± 3.8 to $15.8 \pm 3.2\%$) tended to be greater than in IT (11.5 ± 3.7 to $14.5 \pm 3.4\%$), but the difference did not reach statistical significance ($p = 0.13$). The increase in the endothelial-independent vasodilation (sublingual nitroglycerine) was significantly greater ($p = 0.02$, OHA vs. IT) in the OHA (8.7 ± 2.5 to $18.2 \pm 3.0\%$) than in the IT group (11.2 ± 3.6 to $15.0 \pm 4.0\%$).

Skeletal Muscle Inflammatory Proteins (Figures 1-2)

The protein content of I κ B α , which provided an indirect measure of NF κ B activity, was similar in skeletal muscle samples obtained from OHA and IT groups at baseline, and there were no significant changes in the I κ B α protein content in the muscle after either 30 min or 180 min of insulin infusion in either group before and after 6 months of treatment. There were no significant changes in the mRNA expression of genes regulated by NF κ B (MCP1 and SOD2) in the OHA and IT groups. Corrected p-ERK1/2 protein content at baseline and after therapy (in fasting state and during insulin infusion) was similar in OHA and IT groups. Under fasting conditions corrected p-JNK content was similar in both OHA and IT at baseline and did not change in any group after 6 months of therapy.

Discussion

This study was designed to compare 6 months of equivalent metabolic control with either combination oral hypoglycemic agents versus intensive insulin therapy in adult type 2 diabetic patients of Mexican American ethnicity on vascular and metabolic parameters, CIMT, circulating inflammatory biomarkers and skeletal muscle tissue expression and activity of intermediates involved in inflammation. Both therapies safely and effectively reduced the markedly elevated HbA_{1c} levels and, the combination OHA containing two insulin sensitizing agents (pioglitazone and metformin) was accompanied by $\sim 20\%$ reduction in plasma insulin, a 37% decrease in fasting plasma FFA concentration, suppression of plasma FFA by insulin, and an increase ($\sim 38\%$) in TGD during the insulin clamp. Further, CIMT decreased significantly from 0.080 to 0.068 cm during combination OHA, whereas there was no change with insulin therapy. Although statistically non-significant, FMD was twice as great ($\sim 6\%$ vs. $\sim 3\%$) with OHA vs. IT, and endothelial-independent vasodilation was significantly greater in OHA than in IT. Plasma adiponectin increased in both groups, but more than doubled after OHA and increased only by $\sim 35\%$ in IT. Thus, the possibility that greater elevations in circulating adiponectin levels were responsible for better vascular responses in subjects treated with OHA vs. IT cannot be entirely rule out. Changes in the pro-inflammatory biomarkers in the circulation and in skeletal muscle however were identical 6 months after either therapeutic approach.

Although the number of subjects in each group is relatively small, and the final HgbA_{1c} was slightly lower in OHA (~6.4%) than in IT (~7.1%) these results suggest that oral agents have a strong and reproducible effect on vascular reactivity and that this beneficial effect can be detected within 6 months. Despite a comparable improvement in glycemic control with insulin (HgbA_{1c} = 3.9% in OHA and 3.6% in IT), similar beneficial effects on vascular reactivity, CIMT and insulin sensitivity could not be demonstrated. Because of the small sample size and the short duration of therapy, it remains to be determined whether these differences will persist over a longer period of time and eventuate in decreased CV events. Further confirmation of these vascular findings will be required, especially in view of the fact that measurements are affected by larger errors related to the site, specific protocols, techniques and slow changes with time. Delaying the progression of CIMT (14,23) and a decrease in coronary plaque volume (24) with pioglitazone monotherapy have been demonstrated previously. Although the ProActive (25) and a meta-analysis of pioglitazone studies (26) suggest that this thiazolidinedione may decrease CV events the predictability of the regression of CIMT has not been confirmed in a recent large meta-analyses report (28). In contrast, recent studies with intensive therapy, including insulin administration (6, 7) have failed to demonstrate a reduction in CV events. Our observation that adiponectin, which possesses some anti-atherogenic properties also increased to a greater extent in the OHA than in IT, further supports the reported reductions in CV events when oral therapy, including thiazolidinedione is used in T2DM.

Muscle biomarkers of inflammation, however either did not change or changed similarly in both groups. It is noteworthy that our measurements were made in biopsies obtained from skeletal muscle and not from arterial tissue, which may in part explain the dichotomy between the vascular responses and the observed changes in tissue pro-inflammatory intermediates demonstrated in this study. The greater increase in plasma HDL-cholesterol in the OHA group may have particular relevance to the regression of carotid IMT since a recent study by Davidson et al (27) demonstrated that plasma HDL-cholesterol was the best correlate of carotid IMT changes in T2DM patients treated with pioglitazone. However, it is very difficult to assess the contribution of non-atherosclerotic components, such as water content of the plaque to these CIMT image changes. Lastly, some comment about the marked reduction in plasma FFA concentration in the OHA group is warranted. We (29) and others (30) have shown that acute elevation in plasma FFA concentration induces insulin resistance, impairs endothelial-function and activates inflammatory pathways in muscle. One would expect that the converse, i.e., a reduction in plasma FFA, would have the opposite effect on these vascular and metabolic parameters in T2DM patients.

In conclusion, this study demonstrates that 6 months of intensive therapy with either OHA or IT produces a comparable reduction in HgbA_{1c} in poorly-controlled Hispanic T2DM patients. However, despite comparable glycemic control, only OHA therapy increased insulin sensitivity and endothelial function and reduced CIMT. These findings, albeit in a relatively small group of subjects, indicate that OHA may have significant CV and metabolic advantages over insulin therapy. The design of this study does not permit a clear dissection of which of the oral agents is responsible for the beneficial CV and metabolic effects. There is little evidence to suggest a role for the sulfonylurea, whereas insulin

sensitizers, both pioglitazone (14, 24-26) and metformin (5) have been demonstrated to enhance vascular endothelial function and to exert anti-atherogenic effects.

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Abbreviations

ASCVD	Atherosclerotic cardiovascular disease
T2DM	type 2 diabetes mellitus
CV	Cardiovascular
CIMT	Carotid Intimal Media Thickness
TDI	Texas Diabetes Institute
OHA	Oral Hypoglycemic Agents
IT	Insulin Therapy

Figure 1.

A) $I\kappa B\alpha$ protein content in skeletal muscle prior to and after insulin therapy (IT) and oral hypoglycemic agents (OHA) therapy following an overnight fast (baseline) and 30 and 180 minutes after insulin infusion during the euglycemic insulin clamp.

B) Effect of IT and OHA on MCP1 mRNA expression in human skeletal muscle

C) Effect of IT and OHA on SOD2 mRNA expression in human skeletal muscle.

Figure 2.

A) Changes in ERK1/2 phosphorylation corrected to total ERK1/2 protein content in human skeletal muscle before (B) after (A) 6 months of treatment after an overnight fast (baseline) and 30 and 180 minutes of insulin infusion during the euglycemic insulin clamp (p=NS)

B) Effect of insulin therapy (IT) and oral hypoglycemic agent (OHA) on JNK1/2 phosphorylation corrected to total JNK1/2 protein content in human skeletal muscle following an overnight fast, before (B) and after (A) 6 months of treatment

Table 1
Subject characteristics at baseline and after 6 months of therapy with either hypoglycemic agents (OHA) and with combination glargine-apidra insulin therapy (IT)

Characteristics	OHA (n=9)		p@		IT (n=12)		p@
	Before	After			Before	After	
Age (yrs)		44±3				46±9	
Gender (M/F)		(6/3)				(5/7)	
T2DM (years)*		3.7±1.2				6.9±1.6	
Weight (kg)*	94.9±5.9	102.4±5.0	0.01		83.8±4.8	88.0±5.0	0.01
BMI (kg/m ²)*	32.6±1.8	36.7±1.9	0.01		32.5±1.6	34.2±1.7	<0.01
Waist (cm)	106.8±4.3	111.8±3.9	0.08		96.9±10	109.2±3.6	0.16
Hips (cm)	114.3±4.2	117.5±4.1	0.31		111.3±3.4	113.3±4.1	0.44
Fat %	33.7±2.9	35.2±2.9	0.02		37.5±2.4	37.8±2.3	0.05
Fat (kg)*	31.3±3.6	35.6±4.0	0.04		30.8±2.8	33.1±2.9	0.001
Lean mass (kg)	57.6±3.6	58.8±3.4	0.38		48.2±2.7	49.7±2.8	<0.001
FPG (mmol/l)	14.28±0.93	6.89±0.61	<0.01		14.22±0.94	7.89±0.60	<0.01
Hgb _{A1C} %	10.3±.7	6.4±0.2	<0.01		10.7±0.6	7.1±0.3	<0.01
SBP (mmHg)	127±6	134±5	0.19		140±3	133±3	0.06
DBP (mmHg)	82±1	81±2	0.30		81±4	75±3	0.19
Medications							
ACE	4				3		
ARBs	1				0		
Thiazides	3		NS		1		NS

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Characteristics	OHA (n=9)		p [@]		IT (n=12)		p [@]
	Before	After	Before	After	Before	After	
Statins	1				1		
Fibrates	1				0		
Aspirin	1				0		

[@] = Before vs. After and

* = p<0.05 OHA vs. IT (see text for details); BMI (body mass index); FPG (fasting plasma glucose); SBP = systolic and DBP diastolic blood pressure; NS=non-significant, refers to all medications.

Table 2
Plasma lipids and inflammatory biomarkers at baseline and after 9 months of therapy with either hypoglycemic agents (OHA) and with combination glargine-apidra insulin therapy (IT)

Parameters	OHA (n=9)		p@	IT (n=12)		p@
<i>Plasma Lipids</i>	Before	After		Before	After	
Total Cholesterol (mg/dl)	184.3±13.7	182.7±8.5	0.88	181.4±13.0	165.2±11.4	0.06
LDL-cholesterol (mg/dl)	103.3±6.6	109±7.1	0.60	114.1±13.6	106.1±8.5	0.08
HDL-cholesterol (mg/dl)	39.4±5.3	43.6±5.0	0.04	36.3±2.1	40.0±3.4	0.24
Triglycerides (mg/dl)	225.5±103.6	167.8±36.4	0.48	188.2±40.7	171.6±35.7	0.56
<i>Particle Size</i>						
VLDL (nm)	59.4±2.5	55.7±1.7	0.03	56.1±2.8	53.8±1.7	0.56
HDL (nm)	8.7±0.2	8.6±0.2	0.1	8.5±0.1	8.7±0.2	0.14
LDL (nm)	20.7±0.3	20.9±0.3	0.37	20.3±0.3	20.39±0.3	0.65
<i>Inflammatory Markers</i>						
hs-CRP (µg/ml)	4.3±0.9	3.4±1.0	0.47	4.1±1.2	4.9±1.5	0.73
Adiponectin (µg/ml) *	6.6±1.2	15.3±1.9	0.002	6.4±1.0	9.9±1.1	0.004
Leptin (ng/ml) *	11.5±2.2	21.6±7.0	0.06	21.2±5.8	29.7±7.8	0.01
ICAM (ng/ml)	225.0±16.1	225.5±26.4	0.97	229.6±16.8	231.9±22.1	0.87
VCAM (ng/ml)	431.8±36.9	412.5±33.9	0.65	453.5±25.8	484.3±35.9	0.45
IL-6 (pg/L)	2.6±0.3	2.3±0.4	0.43	2.9±0.3	3.0±0.4	0.86
TNF-α (pg/ml)	1.4±0.2	1.2±0.1	0.05	1.5±0.4	1.5±0.3	0.83

@ = Before vs. After and

* = p <0.01 OHA vs. IT (see text for details); hs-CRP = high sensitivity C-reactive protein; ICAM and VCAM = intra-cellular and vascular adhesion molecules; IL-6 = interleukin-6; TNF-α = tumor necrosis factor alpha.

Table 3

Plasma free fatty acid concentration and glucose kinetics calculated from the euglycemic hyperinsulinemic clamp performed with tritiated glucose at baseline and after 6 months of therapy with either hypoglycemic agents (OHA) and with combination glargine-apidra insulin therapy (IT)

Parameters	Oral		p@	Insulin		p@
	Before	After		Before	After	
EGP (Basal)mg/kg/min	2.10±0.2	1.77±0.2	0.07	2.14±0.26	1.97±0.23	0.27
EGP (clamp) * mg/kg/min	0.42±0.18	0.37±0.16	0.42	0.47±0.12	0.66±0.20	0.53
FFA (fasting) * mEq/L	620.8±77.4	392.5±38.9	<0.01	480.2±50.6	520.1±40.5	0.29
FFA (clamp)mEq/l	152.2±31.3	54.8±15.4	0.01	150.2±30.8	110.2±20.3	0.14
Insulin (fasting)µU/ml	8.04±1.57	6.96±1.08	0.2	12.55±4.19	14.34±4.11	0.24
TGDMg/Kg/min	3.45±0.61	5.31±0.68	0.03	3.72±0.33	4.67±0.46	0.07
[100 × (TGD/I)] * mg/Kg/min/µU/ml	5.2±0.5	8.3±0.8	0.03	6.0±0.9	4.7±0.5	0.12

@ = Before vs. After and

* = p<0.05 OHA vs. IT (see text for details); EGP = endogenous glucose production during *basal* and steady-state *clamp* period; FFA = plasma free fatty acid concentration at *fasting* and during steady-state *clamp* period; TGD = total glucose disposal; and TGD/I = total glucose disposal corrected for the achieved steady-state plasma insulin concentration during the clamp procedure.

Table 4
Results of the vascular function tests and carotid intimal media thickness (CIMT) measurements at baseline and after 6 months of therapy with either hypoglycemic agents (OHA) and with combination glargine-apidra insulin therapy (IT)

Vascular Tests	Oral		Insulin	
	Before	After	Before	After
<i>Reactive Hyperemia</i>			p@	p@
% Diameter Increase	9.8±3.8	15.8±3.2	0.13	14.5±3.4
% Blood Flow Increase	66.9±25.0	70.2±17.4	0.46	86.0±19.3
<i>SL Nitroglycerin</i>				
% Diameter Increase *	8.7±2.5	18.2±3.0	0.02	15.0±4.0
% Blood Flow Increase	33.7±10.8	52.0±10.4	0.04	59.8±23.2
CIMT (cm) *	0.080±0.004	0.068±0.003	0.03	0.072±0.006

@ = Before vs. After and

* = p<0.05 OHA vs. IT (see text for details); CIMT = carotid intimal media thickness; SL sublingual.