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Research Article

Remission of Type 2 Diabetes, Decreased Inflammation and Cardiovascular Risk Factors in Obese Adults: Randomized Control Trial

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Abstract

Objective: Remission of type 2 diabetes (T2D) to normal glucose tolerance (NGT) has had little success in the past. Objectives of this study were to determine the effect on remission of T2D using a high protein (HP) vs. high carbohydrate (HC) diet and effects on inflammation, oxidative stress, cardiovascular risk factors (CVR), metabolic parameters and body mass (% lean and fat) in obese subjects with T2D after dietary intervention for 6 months.

Research Design and Methods: Women and men (12) with T2D were recruited and randomized to either a HP (30% protein, 30% fat, 40% carbohydrate) (n = 6) or HC (15% protein, 30% fat, 55% carbohydrate) (n = 6) diet for the 6 months feeding controlled trial. All food was purchased at local grocery stores and provided to subjects for 6 months with daily food menus for weekly food pick-up for compliance of the respective HP and HC diets and weekly weight measurements. Oral glucose tolerance and meal tolerance tests with insulin and glucose measurements and DXA scans were done at baseline and after 6 months on the respective diets.

Results: 100% of the HP subjects had remission of T2D to NGT, but only 16.6% of subjects on the HC diet had remission of T2D at 6 months. HP diet group had significant improvement in a) insulin sensitivity ($p = 0.001$), b) inflammatory cytokines ($p = 0.001$), c) cardiovascular risk factors ($p = 0.004$), d) oxidative stress ($p = 0.001$), e) increased % lean body mass ($p = 0.001$) compared to the HC diet group at 6 months.

Conclusion: 100% remission of T2D to NGT with the HP diet and significant improvement in inflammation, metabolic parameters and CVR was achieved compared to HC diet at 6 months.

Keywords: *Type 2 Diabetes Remission; Insulin Sensitivity; Inflammation; High Protein Diet; Weight Loss; Cardiovascular Risk Factors*

Introduction

The prevalence of people with type 2 diabetes mellitus (T2D) has continued to increase over the past 20 years with currently approximately 37 million people with diabetes, 27 million who are diagnosed and 7.2 million who are undiagnosed in the USA according to the Center for Disease Control (CDC) 2022 National Diabetes Statistics Report [1]. One of the highest risk factors for T2D, as well as heart disease, hypertension and other metabolic disease is obesity [2]. Obesity has reached epidemic proportion in the USA with over 42% adults

estimated to be obese [3,4]. There is a 93 fold increase in T2D as BMI increases from 23 to > 35 kg/m^2 [4,5]. The rate of conversion for impaired glucose tolerance (IGT) (prediabetes) to T2D is between 7- 10%/year and no difference in ethnicity has been shown by diabetes prevention program (DPP) [6,7] and ACT NOW [8] studies. Subjects with diabetes are at increased risk of numerous medical and complications such as seen in COVID-19 subjects with diabetes have had a higher incidence of hospitalization and mortality [9-11]. DPP [6,12] and other similar diabetes prevention studies [13,14] have shown the importance of diet in reducing the risk of conversion of IGT to T2D. Attempts to reduce the risk of T2DM, complications and medical costs must start in the early part of diagnosis [15-17]. Various diets have been recommended for T2D [18-22] and non- diabetics and proposed potential advantages of low-carbohydrate [23,24] or high- protein diets [25,26], but there is not a consensus on a diet to manage blood glucose and weight loss in T2D subjects [27]. Also, there has not been established a diet for weight loss and glucose control and converting from T2D to Normal Glucose Tolerance (NGT). Given the destructive metabolic changes that occur with T2DM it would be prudent to determine if one exists.

Our studies of the effect of a High Protein (HP) diet or High Carbohydrate (HC) diet on IGT obese subjects [28] and NGT obese subjects [29] demonstrated similar weight loss of 9-10% in both diet interventions, but greater advantages of the HP diet for insulin sensitivity (100% remission of IGT to NGT), reduced CVR factors, oxidative stress (ROS), and inflammatory cytokines (IC) [28,29]. HP intake has the potential to suppress hunger and induce satiety [18,25,30] with a negative relationship between protein content and glycemic index [31,32]. Increased protein intake has also been shown to reduce energy intake independent of the effect of satiety [25]. Proteins have about a 30 percent thermic effect of feeding [33] by increasing protein digestion and synthesis which is much higher than carbohydrates or fats. Diet composition can affect the lipid profile and its metabolism [34-37] and lipids are considered a primary risk factor for CV disease. Triglycerides decreased significantly more in our studies with the HP diet than the HC diet [28,29] demonstrating that increasing protein in the diet may beneficially change the lipid profile. It has been shown that protein intake induces insulin release and is different in non-diabetic and diabetic individuals [38]. Protein is a less potent secretagogue for insulin than glucose in normal individuals [39] as demonstrated in our studies showing a lower insulin response to HP than HC diet [28,29]. This suggests that HP diets could help preserve the Beta cells and decrease insulin load per meal with increasing insulin sensitivity. Our studies showed an increased release of the incretins, GLP-1 and GIP, with the HP diet compared to the HC diet [40,41]. Treatment with DPP-4 inhibitors and GLP-1 receptor agonists and have been shown to improve insulin sensitivity and cardiovascular risks in T2D subjects [27,42-48]. With the increase in GLP-1 and GIP observed in our studies, the HP diet may be beneficial in treating T2D and CVR.

Other important aspects of the HP diet are the decreased glucose area under the curve (AUC) and the anti-inflammatory effect compared to the HC diet [28,29]. Studies have demonstrated that elevated glucose or Free Fatty Acids (FFA) lead to activation of leukocytes and increase in cytokines and reactive oxygen species (ROS) [49-52].

Studies have shown that hyperglycemia in T2D and obesity is associated with increased inflammatory cytokines [53,54]. Thus, reduction in inflammatory cytokines in diabetes is important in view of the fact that patients who develop acute respiratory distress [55-58] and COVID-19 have increased inflammatory cytokines, and diabetes would add to this inflammatory effect [59].

Protein content in the diet may also help maintain lean body mass [28,60,61]. Few studies have compared diets with adequate percentages of macronutrients and sufficient follow-up time where high protein is compared to high carbohydrate diets and determined effects on CVR factors, inflammatory cytokines, ROS [28,29,52,53,62-66] especially with respect to subjects with type 2 diabetes.

Therefore, this study was designed to determine if remission of T2D in newly diagnosed patients with T2D could be obtained with a tightly controlled HP or HC diet feeding study and quantitate longitudinally the changes in various metabolic markers of insulin sensitivity, inflammatory cytokines, CVR factors, ROS, and changes in muscle and fat mass and weight loss from baseline to 6 months.

Research Design and Methods

Patients

Women and men age 20 - 60 years old with a BMI ≥ 30 kg/m^2 to ≤ 55 kg/m^2 diagnosed with T2D within the past two years or diagnosed at this study screening visit were recruited for the study. Inclusion criteria for subjects was age, BMI, fasting glucose of ≥ 126 mg/dl, 2 hour glucose level of ≥ 200 mg/dl during a standard oral glucose tolerance test (OGTT), and HbA1c of 6.5 - 10%. Subjects were excluded if they had elevated serum creatinine (> 1.5 mg/dl) or proteinuria, abnormal liver function tests, on antidiabetic agents or insulin, thyroid disease with abnormal TSH, weight > 350 lbs, LDL cholesterol > 160 mg/dl, triglycerides > 400 mg/dl, SBP > 145 or DBP > 100 mm, on medications known to effect glucose or lipid metabolism, pregnancy or desire to become pregnant in the next 6 months, weight loss of more than 5% of body weight in the last 6 months, history of cancer undergoing active treatment or smoked. Subjects who were on metformin who wanted to participate in the study were discontinued from the drug by their PCP and baseline tests were performed on these subjects 4 weeks after discontinuing the drug. Subjects meeting the above criteria were asked to keep a food diary for a week to determine food likes and ability to keep a daily diet diary.

Of the 83 subjects screened by phone 26 were asked to sign the consent form and testing for meeting the inclusion criteria. Fifteen of these subjects met all the inclusion criteria testing and were randomized to a HP diet (7 subject) vs. HC diet (8 subjects) for a period of 6 months. One subject in the HP group and 2 subjects in the HC group dropped out within a few weeks after screening due to their work schedule, driving distance or moved to different area. Since only baseline data was available, not (OGTT, MTT, DXA, weight, metabolic markers) for 6 months, those subjects that dropped out were not included in the data analysis of comparison of changes from baseline to 6 months on the diet interventions. Therefore, six subjects in each group completed the 6 month study as shown in figure 1 and data analyzed.

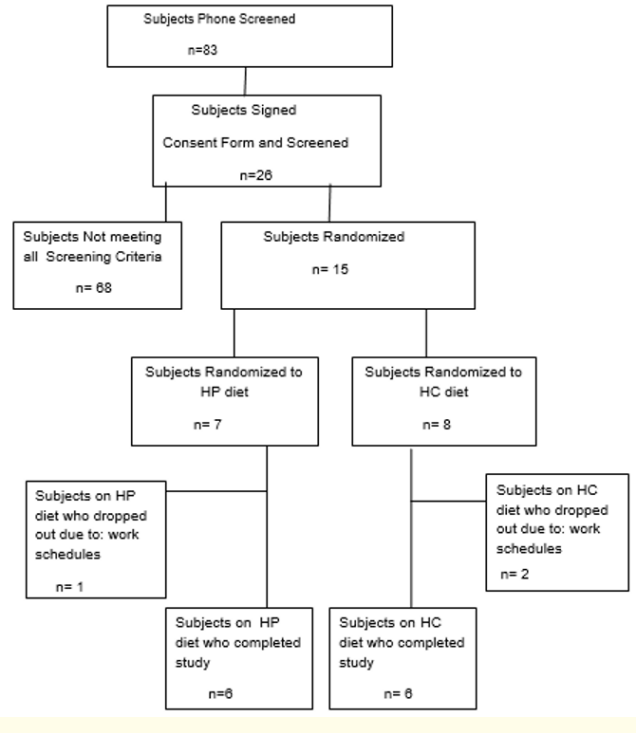

Figure 1: *Shows the recruiting and screening of subjects for the participants in the study.*

Study design

The study was a prospective, randomized trial to study remission of type 2 diabetes (T2DM) and effects on inflammation, CVR, oxidative stress and other metabolic parameters using a high protein (HP) diet (30% Kcals protein, 40% Kcals of carbohydrate (CHO), 30% Kcals of fat) compared to a high carbohydrate (HC) diet (15% Kcals of protein, 55% Kcals of CHO, 30% Kcals of fat) for a period of 6 months. The study was approved by the Institutional Review Board of the University of Tennessee Health Science Center (UTHSC).

All participants visits were at the General Clinical Research Center (GCRC) at UTHSC. After signing the consent form, height and weight, blood pressure, waist measurements and history and physical examination were done. A standard OGTT and mixed meal tolerance test (MMT) after an overnight fast were done on the subjects at baseline (Bl) and 6 months of the study. The MTT for the HP group was a high protein meal and the MTT for the HC group was a HC meal. HC and HP meals were 300 calories (the same number of calories as the 75 gm OGTT). Blood was drawn at baseline and 30 minutes intervals for 2 hours for measurements of glucose and insulin in order to determine remission of T2D, insulin sensitivity and glucose response. RMR resting metabolic rate (RMR), DXA scan, chemistry profile, lipid profiles, complete blood count (CBC), parathyroid hormone (PTH), vitamin D, cytokines, ROS, 24 hour urine collections (for microalbumin, calcium (Ca) and urinary urea nitrogen (UUN) and creatinine clearance (CrCl)) were all done at baseline and 6 months for determination of inflammation, CVR, ROS, changes in body weight and body composition (lean and fat mass), calcium metabolism and protein breakdown (by urinalysis). Assessment of subjects for level of physical activity showed all were at minimum activity at baseline.

Per the American Diabetes Association (ADA) [27] recommendation of 150 minutes of exercise/week, patients were asked to walk 30 minutes/day and were given FitBits to monitor their level of physical activity which was monitored weekly throughout the study. A permuted block randomization method generated by the biostatistician was used for randomization of subjects to either the HP or HC diet after meeting the screening criteria.

Subjects who had a fasting glucose of < 100 mg/dl and 2 hour glucose level of < 140 mg/dl during a OGTT and HbA1c ≤ 5.7% at 6 months were considered to have remission of T2D to normal glucose tolerance (NGT). They were considered to have remission to prediabetes if they had a fasting glucose of 100 to < 126 mg/dl, and 2 hour glucose level of 140 to 199 mg/dl during the OGTT and HbA1c > 5.7 - 6.4% at 6 months. Subjects who at 6 month OGTT had a fasting glucose of ≥ 126 mg/dl and 2 hour glucose level of ≥ 200 mg/dl and HbA1c ≥ 6.5% did not have remission of T2DM and were referred back to their Primary Care Physician (PCP) or Endocrinologist for pharmaceutical treatment. Subjects who had remission of T2DM were transitioned to purchasing their own food and meal preparations using diet plans they were using during the study when all food was provided. These subjects were followed for an additional 6 months by phone, emails and dietary consultation to help them maintain their remission of diabetes and weight loss.

Diet related parameters

Resting metabolic rate (RMR) was performed on an individual basis to determine caloric maintenance needs after which 500 Kcals/day was subtracted from the maintenance caloric needs to promote a 1 - 2 lbs weekly weight loss. To achieve adequate weight loss on average a 1700 Kcal/day diet for a 100 kg subject was used with no subject on less than 1200 Kcal/day.

In order to ensure accurate macronutrient consumption a feeding study where all food and daily menus were provided was necessary. The meals were distributed in pre- packaged foods of 3 meals a day plus snacks by the dietician affiliated with the UT CRC dietary services in order to maintain the macronutrient and caloric requirements established at randomization. All food was provided for the entire 6 months with weekly food to pick up and daily menu food records for the week and weight measurements.

For compliance of diet adherence food records were required to be returned at the next food pick up [67].

All food menu plans met the recommended daily intake of minerals and vitamins for women and men age 20 - 60 years as assessed by the University of Minnesota Nutrition Data system and were available at local grocery stores. The HP and HC diets contained more than the recommended 1000 mg/day daily intake (RDI) of calcium for women and men 20 - 60 years of age [68]. Since these T2D subjects were obese and possibly at risk for coronary heart disease, it was important to ensure the subjects follow a healthy diet that would minimize health risks. The food dietary fat sources mostly contained monounsaturated and polyunsaturated fats [63,69,70]. The dietary protein sources included fish, chicken, lean meats, eggs and non-fat dairy foods and carbohydrate sources emphasized whole grains, fruits, vegetables and legumes. Food menus were consistent with the guidelines of the American Diabetes Association [27] and Institute of Medicine [69].

Body composition by DXA, resting metabolic rate (RMR)

Hologic discovery QDR bone densitometer (version 8.4) (DXA) measurements were done at baseline and 6 months to measure body-composition including lean mass (LM), fat mass (FM), and bone mineral content (BMD) [28].

To assess RMR of each participant indirect calorimetry using a Cardio Coach (Korr Medical Technologies) was done at the beginning of the study to determine the caloric diet intake needs for weight loss for each subject and at 6 months to determine changes in their metabolic rate as we previously have described [28,71].

Laboratory procedures

Glucose and insulin levels for the OGTT and MTT for baseline and 6 months, were measured at 0, 30, 60, 90, and 120 minutes and AUC calculated using the Trapezoidal rule. Insulin, glucose, inflammatory cytokines (TNFa, IL-1β, IL-6, INFɣ, MCP-1), CVR factors (blood pressure, triglycerides, LDL, HDL, cholesterol, BMI, hcCRP, FFA), adiponectin, oxidative stress (ROS) (dichlorofluorescein (DCF) and MDA), β- hydroxybutyrate, HbA1c were measured using our previously established methods [28,29,53,57,58,72]. The Coefficient of Variation of the assays were all less than 5 percent.

Chemistry metabolic profile (CMP), CBC, TSH, cortisol, urine urea nitrogen (UUN) and other tests to exclude chemical and metabolic abnormalities and protein balance were determined by standard clinical lab procedures. Muscle mass catabolism and protein were assessed via 24-hour UUN, CrCl and Ca at baseline and 6 months.

Calcium balance was assessed by 24 hr urine Ca excretion as well as serum Ca, PTH, and 25 OH-vitamin D at baseline and 6 months. Waist and BMI were measured by standard methods.

Insulin sensitivity and beta cell function

The homeostasis model assessment was used to determine the insulin resistance HOMA IR [73]. Insulin sensitivity (ISI) was determined from plasma insulin and glucose levels from the OGTT using the Matsuda insulin index [8,74]. Beta cell function was calculated as previously described from plasma glucose and insulin measurements obtained during the 2 hour OGTT [8,28,29].

Statistical analysis

The primary outcomes analyzed were remission of type 2 diabetes, markers of insulin sensitivity, inflammatory cytokines, cardiovascular risk factors, and change in lean and fat body mass from baseline to 6 months. Change in effects between the two HP and HC diets was compared using Wilcoxon rank sum test. To compare the effects of each diet from baseline and 6 months Wilcoxon signed rank test was used. A p- value less than 0.05 was considered statistically significant.

Results

HP and HC groups were not statistically different at baseline (Bl). Table 1 shows the mean ± SE of various parameters analyzed on the twelve (6 HP and 6 HC) diet subjects at Bl to 6 months (mo) and the significant changes in these parameters on the HP diet from Bl to 6 mo and HC diet from Bl to 6 mo. The last column shows the significant difference between the HP vs HC diet at 6 mo. Of significant importance is the 100% (6/6) remission of T2DM to normal glucose tolerance (NGT) in all the HP diet group subjects; whereas, there was only a 16% (1/6) remission to NGT and (1/6) remission to IGT in the HC group. Both the HP and HC subjects had significant weight loss at 6 mo from Bl weights but not significantly different loss between the HP and HC groups at 6 months. The waist measurements decreased from 112.5 ± 3 cm at Bl to 105.2 ± 2 cm at 6 months (p = 0.001) in the HP diet group and in HC diet group decreased from 110.7 ± 3.4 at Bl to 103.5 ± 4 at 6 months (p = 0.005). HbA1c was significantly improved to normal range with the HP group whereas, the average HbA1c did not decrease below the criteria level of T2D in the HC diet group after 6 mo. Insulin sensitivity (HOMA IR and ISI) as shown in table 1 and Beta cell function which increased from Bl (3.1 ± 0.3) to 6 mo (11.1± 2.1) (p = 0.001) in the HP diet group and from Bl (3.2 ± 0.3) to 6 mo (5.26± 0.9) (p = 0.03) in the HC group were significantly improved at 6 mo from Bl in both the HP and HC subjects; however, the HP group had significantly greater improvement in these parameters compared to the HC group at 6 mo (p = 0.001). Diet compliance was 94.7% and 94.1% for the HP and HC diet groups, respectively, which were not significantly different. Table 2 shows the inflammation markers (TNFα, IL-1β, IL-6, IFNɣ, MCP-1, hsCRP) and CVR factors (BP, cholesterol, triglycerides, LDL, HDL), and oxidative stress markers (ROS (DCF, MDA) were significantly decreased in both diet groups. The FFA were significantly decreased in the HP diet group at 6 months but not in the HC diet group. The HP diet subjects had significantly greater reduction in these inflammatory cytokines, CVR factors and oxidative stress markers compared to the HC subjects at 6 mo. This decrease in TNFα, IL-1β, IL-6, IFNɣ, MCP-1, and hsCRP elucidates a better anti-inflammatory effect of the HP diet compared to the HC diet.

	HP (n = 6)			HC (n = 6)			
Parameters	**Baseline**	**6 months**	**p***	**Baseline**	**6 months**	**p***	**p****
Female/Male	4/2			4/2			
Ethnicity AA/C	3/3			3/3			
BMI (kg/m^2)	39 ± 1.8	36 ± 1.9	< 0.001	36 ± 1.7	33 ± 1.6	0.002	0.391
Weight Loss (lbs)		15.4 ± 2.5	< 0.001		19.5 ± 2.2	< 0.001	0.692
HbA1c	7.7 ± .05	5.6 ± .02	0.001	7.8 ± .04	6.7 ± .06	0.01	0.002
% Remission of Type 2 Diabetes		100% remission to NGT			16.6% remission to NGT		0.001
Insulin Sensitivity							
HOMA IR	5.3 ± 0.29	2.1 ± 0.13	0.0001	5.2 ± 0.27	4.4 ± 0.26	0.03	0.004
ISI (Matsuda I)	1.4 ± 0.2	6.4 ± 0.9	0.0001	1.5 ± 0.3	3.0 ± 0.4	0.04	0.0001

Table 1: *Changes in insulin sensitivity and metabolic parameters with the HP and HC diets.*

Analysis Mean ± SE were calculated.

***Wilcoxon rank sum test was used to compare variables between the two diet groups at 6 mo and *Wilcoxon signed rank test to compare baseline and 6 mo within diet group. p ≤ 0.05 was consideredstatistically significant.*

	HP (n = 6)			HC (n = 6)			
Parameters	**Baseline**	**6 months**	**p***	**Baseline**	**6 months**	**p***	**p****
Inflammation							
TNFα (pg/ml)	19.03 ± 3.53	3.9 ± 0.71	0.005	17.75 ± 1.62	12.6 ± 1.9	0.05	0.001
IL-6 (pg/ml)	10.6 ± 0.41	4.7 ± 0.5	0.005	10.57 ± 0.31	9.1 ± 0.9	0.07	0.005
IL-1β (pg/ml)	14.7 ± 0.48	3.1 ± 0.73	0.001	14.5 ± 0.51	9.4 ± 0.4	0.03	0.001
MCP-1 (pg/ml)	18.7 ± 2.9	1.8 ± 0.9	0.003	18.1 ± 2.5	13.3 ± 2.1	0.04	0.001
IFN-ɣ (pg/ml)	15.7 ± 0.61	8.7 ± 0.4	0.005	15.9 ± 0.51	12.4 ± 0.7	0.06	0.005
hsCRP (mg/L)	16.05 ± 1.01	2.85 ± 0.31	0.005	16.75 ± 0.8	7.4 ± 0.5	0.01	0.001
Cardiovascular Risk Factors (CVR)							
BP (sys/diast)	128.3/85.3 ± 3/2	117/78.8 ± 2/2	.01/.01	129/85 ± 3/2	117/79 ± 3/3	.01/.01	.73/.77
Cholest (mg/dl)	176 ± 14	152 ± 10	0.01	185 ± 13	170 ± 9	0.02	0.02
TG (mg/dl)	139 ± 14	95.8 ± 10	0.01	155 ± 13	159 ± 9	0.06	0.02
HDL (mg/dl)	49 ± 2	52 ± 2	0.04	49 ± 3	50 ± 2	0.08	0.05
LDL (mg/dl)	94.5 ± 4.2	80.4 ± 3.7	0.01	98 ± 4.4	95 ± 4.0	0.07	0.01
FFA (mmol/L)	0.73 ± 0.05	0.43 ± 0.03	0.001	0.71 ± 0.04	0.79 ± 0.03	0.04	0.001
Oxidative Stress (ROS)							
DCF (μM)	4.0 ± 0.3	2.5 ± 0.1	0.01	4.1 ± 0.3	3.5 ± 0.3	0.04	0.01
MDA (μM)	2.0 ± 0.09	0.8 ± 0.06	0.01	2.1 ± 0.08	1.5 ± 0.08	0.04	0.03

Table 2: *Changes in inflammatory, CVR and oxidative stress markers with the HP and HC diet.*

Analysis Mean ± SE were calculated.

***Wilcoxon rank sum test was used to compare variables between the two diet groups at 6 mo and *Wilcoxon signed rank test to compare baseline and 6 mo within diet group. p < 0.05 was considered statistically significant.*

Figure 2A shows the mean ± SE of glucose values for the OGTT for the HP and HC diet groups. Bl HP vs HC glucose response as shown by the figure and area under the curve (AUC) for the OGTTs are not significantly different. OGTT glucose response and AUCs for the HP and HC diet groups were significantly less at 6 mo than at Bl (HP Bl vs HP 6 mo (glucose p = 0.0005), HC Bl vs HC 6 mo (glucose p = 0.01).

However, the OGTT glucose response AUCs for the HP diet were significantly less than the HC diet at 6 months (p = 0.0001) showing a greater improvement in glucose disposal with the HP diet than the HC diet. Figure 2B shows the mean ± SE for glucose for the MTT for the HP and HC diet groups. The MTT HP vs HC glucose AUCs at Bl were significantly different (p = 0.001) demonstrating the difference in glycemic response to the HP vs HC meal of the same caloric intake (300 kcal). Glucose AUCs for the HP (p = 0.005) and HC (p = 0.01) diets at 6 months were significantly less than at Bl.

However, the glucose AUCs for the HP MTT was significantly less than the glucose of the HC MTT (p = 0.0001) after 6 months on the respective diets. Thus, the HP diet caused a decreased blood glucose level, greater glucose disposal and improved insulin sensitivity.

Figure 2C shows the mean ± SE for the insulin values for the OGTT for the HP and HC diet groups. There was no significant difference of the Bl HP vs HC insulin response to the glucose as can be seen by the figure and area under the curve (AUC) for the insulin. Insulin response and AUCs for the HP and HC diet groups were significantly less at 6 mo than at Bl (HP Bl vs HP 6 mo (p = 0.0005), HC Bl vs HC 6 mo (p = 0.01). The OGTT insulin AUC response for the HP diet was significantly less than the HC diet at 6 mo (p = 0.0001) showing a greater

improvement in insulin sensitivity with the HP diet than the HC diet. Figure 2D shows the mean ± SE for insulin for the MTT for the HP and HC diet groups. The MTT HP vs HC insulin AUCs at Bl were significantly different (p = 0.001) demonstrating the difference in insulin response to a high protein vs high carbohydrate meal of the same caloric intake (300 kcal). Insulin AUCs for the diets at 6 months were significantly less than at Bl (HP (p = 0.001) and HC (p = 0.01)) and the insulin AUCs for the HP MTT were significantly less than the HC MTT (p = 0.0001) after 6 months on the respective diets, demonstrating greater insulin sensitivity and less stress on the B cells for insulin release for the same caloric intake with the HP diet β-hydroxybutyrate was measured as a determination of ketones on fasting blood during the study. No significant difference in β-hydroxybutyrate from Bl to 6 mo and no significant difference between groups was observed demonstrating no significant ketosis induced by the diets. All chemistry profile and CBC parameters were in the normal range at baseline and 6 months. A significant increase in UUN in the HP group from Bl to 6 mo (8.9 ± 2.1 to 17.5 ± 2.3 gm/24 hr) compared to no increase in the HC group (8.8 ± 2.2 to 9.0 ± 1.6 gm/24 hr) verifying the HP group was consuming their HP diet. PTH, 25-OH Vitamin D, CrCl, microalbumin, serum Ca or urinary Ca levels were not changed significantly (data not shown) in either the HP or HC group from Bl to 6 mo.

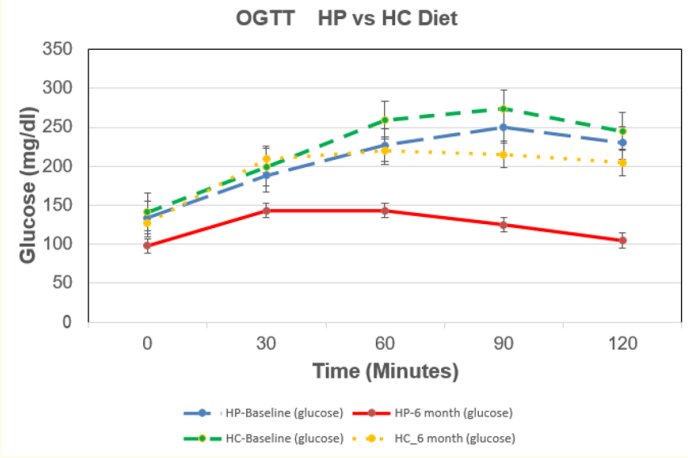

Figure 2A

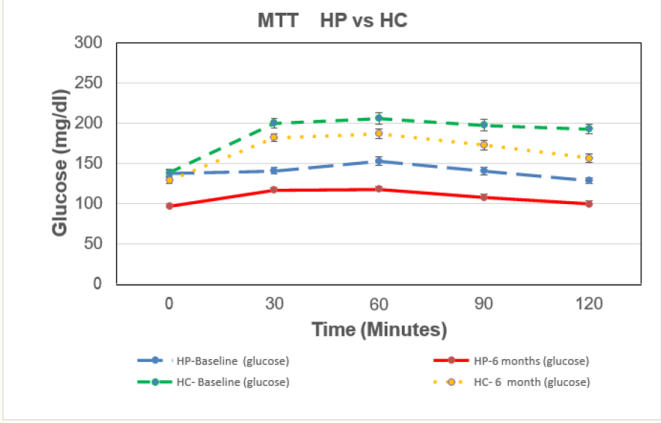

Figure 2B

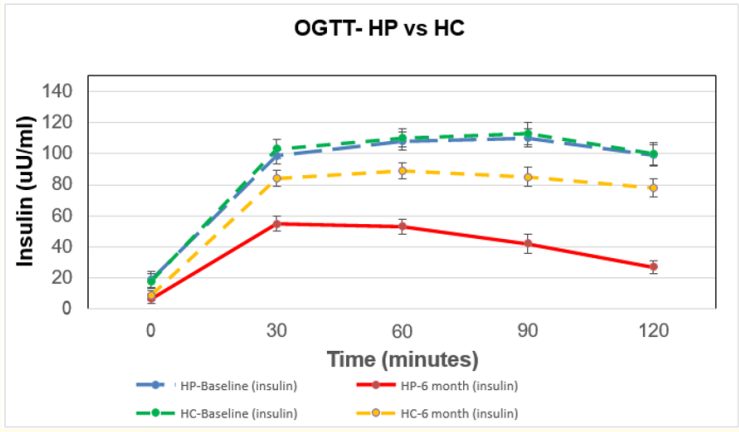

Figure 2C

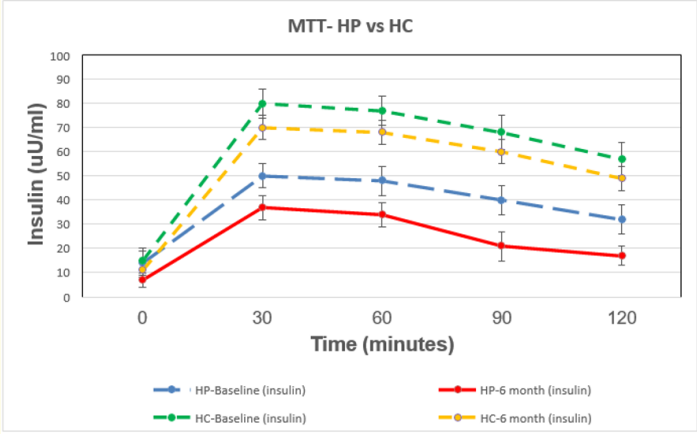

Figure 2D

Figure 2A-2D: *Shows the mean ± SE of glucose and insulin for the 2 hour OGTTs and MTTs for the 6 HP diet subjects and the 6 HC diets subjects. The colored lines represent the following: blue line is HP diet baseline (HP_bl); red line is HP diet at 6 months (HP_6m); green line is HC diet baseline (HC_bl); and yellow line is HC diet at 6 months (HC_6m).*

Figure 3 shows the percent lean mass (LM) and fat mass (FM) loss in the HP and HC groups. The HP subjects had a significant % increase in LM while a significant decrease in % FM from Bl to 6 mo on the HP diet. Whereas, the HC subjects had a significant decrease in both the % LM and FM from Bl to 6 mo on the HC diet, showing that the HP diet group had improvement in LM while achieving overall weight loss.

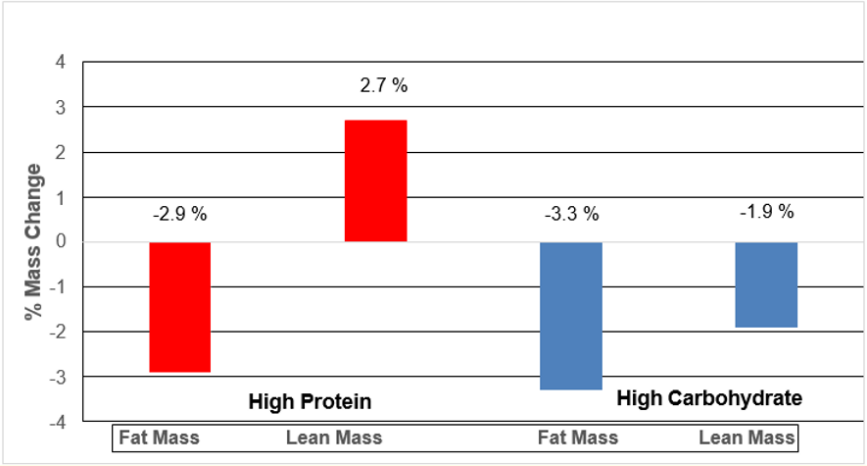

Figure 3 *Shows the effect of the HP and HC diets on percent changes in lean body mass (LM) and fat body mass (FM) at 6 months on the diets.*

Discussion and Conclusion

Important findings of this study are the following: 1) The HP diet resulted in 100% remission of type 2 diabetes to normal glucose tolerance in the subjects while the HC diet resulted in only 16% remission in those subjects. To our knowledge this is the first lifestyle intervention study where remission of T2D has been studied with 100% remission with a feeding study using meals and food obtained from local grocery stores. This study shows that remission of T2D can be achieved with dietary modification if food intake parameters are tightly controlled. 2) The HP diet subjects had greater improvement in insulin sensitivity and greater reduction in inflammation, oxidative stress (ROS) and cardiovascular risk factors compared to the HC subjects. 3) The % lean body mass (LM) increased while % body fat mass (FM) was decreased in the HP diet group; unlike the HC group where both % LM and FM decreased. An important factor in improving insulin sensitivity may be the preservation of % LM in the HP diet group since muscle is a major insulin sensitive tissue for glucose uptake. 4) A high level of compliance (>90%) was obtained by both the HP and HC diet groups by giving the diet meals and menus along with survey of food consumption to the subjects at weekly visits to our CRC.

Since the American Diabetes Association recommends that subjects with T2D exercise 30 minutes per day, all subjects were asked to walk for 30 minutes per day and this was monitored by Fitbits given to the subjects. There was no significant difference in amount of exercise between the HP and HC diet groups; therefore, not affecting the results.

Although the OGTT glucose levels were similar at baseline, the HC group sustained significantly higher glucose levels remaining in the T2DM range compared to the HP diet group which levels decreased to normal glucose levels after 6 months on the diet. The HP MTT had significantly lower glucose levels at baseline and at 6 months than the HC MTT. The increased improvement in insulin sensitivity and Beta cell function with the HP group compared to the HC group likely equates to decreased β cells stress in the HP group. Our study demonstrates that higher sustained elevated glucose levels by ingestion of glucose or high glycemic foods as with the HC diet correlates with increased inflammation and oxidative stress in the HC group compared to the HP group. Antioxidant enzymes are not sufficient to block oxidative stress and inflammation induced by repeated intake of excess energy in the form of high carbohydrate or high fat diets has been

shown [75]. Therefore, the fact that our HP diet had a significantly greater reduction in inflammation, ROS, and cardiovascular risk factor markers than the HC diet in T2D subjects is of significant importance to subjects health.

Our HP and HC diets contained 1500 mg Ca/day, more than the FDA recommended minimum amount of Ca/day [68] and showed no loss of Ca in the urine nor bone loss [76]. In contrast one study reported that a HP diet caused negative calcium (Ca) balance with increased Ca loss in the urine which could indicate adverse affects in the bones [25]. However, our HP diet (30% protein) is at the upper limit of suggested protein consumption range (10 - 30%) and is not in excess to cause a negative Ca balance.

Neither baseline HOMA-IR or ISI (Matsuda Index) were significantly different between the HP and HC groups although the majority of the subjects in our study were African American (AA); although possible limitation in assessment of insulin sensitivity by HOMA-IR with AA has been suggested [77]. However, both methods of assessment showed greater improvement of insulin sensitivity with the HP diet than the HC diet at 6 months.

An important factor in macronutrient composition diet studies is compliance. All food and daily diet plans were provided to each subject in both the HP and HC diet groups at their weekly visit to the CRC along with daily menu consumption survey (which was to be returned each week) for 6 months. This monitoring resulted in greater than 90% diet adherence. Patient's recall food questionnaires of food they ate days to weeks ago used in many studies is generally inaccurate. Unlike weight loss counseling studies which rely solely on subject's self determined food selections (DPP, Look Ahead) [6,78] and the liquid diet reported by Lean [79], we provided each subject with an individualized menu based on weight loss needs that map out what they are to eat each day for 6 months using foods obtained from local grocery stores. Our HP diet was 30% protein which is not excessive yet preserves muscle mass while not causing any liver or kidney problems; whereas, the high carbohydrate diet was 15% protein and resulted in loss of % lean body mass.

Our study demonstrates it is possible that strict adherence of dietary intervention can produce reliable and important results and remission ofT2D. Remaining on the HP diet plan another 6 months with phone and email consultations with the investigators when requested and the subjects purchasing their own food subjects maintained their weight loss and normal glucose levels at an additional follow up of 6 months.

The meal plans are adjustable with a variety of choices with different meals each day of the week according to the subject's food likes and dislikes. All foods were commonly available at local grocery stores at a daily cost of around thirteen dollars.

Initial instructions and weekly meal plans could be provided to the subject along with phone and email consultation when needed. The HP diet and instructional support used for this study would thus enable primary care physicians and other diabetes care givers to offer an economical means to achieve nutrition-based weight loss and remission of Type 2 Diabetes in both women and men.

Type 2 diabetes is considered to be an inflammatory disease [80] and as we and others have shown these subjects have inflammatory cytokines as do subjects with ARDS, COVID-19 [81,82] and can exacerbate the inflammation response. Decreasing the inflammatory cytokines with remission of Type 2 Diabetes can help prevent other inflammatory diseases from becoming a cytokine storm of inflammation.

Our HP diet, although isocaloric with the HC diet, demonstrated profound metabolic improvements compared to the HC diet. This study shows improving insulin action and attenuating inflammation can be achieved by changing the dietary macronutrients. This study is unique in that we used a non-pharmaceutical means (HP diet) as we have used in our previous studies of prediabetes remission, to achieve remission of T2DM, weight loss, decreased inflammation, oxidative stress and cardiovascular risk factors.

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Competing Interests Statement

The authors have no competing interests to declare.

Contribution Statement

Author contributions: F.B.S. wrote the manuscript, researched data and contributed to the conception, design, coordinated recruitment and following subjects on the study, C.S. conducted history and physical examinations of subjects, reviewed data and manuscript. D.L., S.T., and J.C. performed some of the laboratory assays, reviewed and edited manuscript and organized patient data. Nutritionists at the UTHSC CRC provided information on diets and provided daily food and menus to participants, Dr. Jim Wan was the biostatistician in charge of statistical analysis. F.B.S. is the guarantor of this work and, as such, had full access to the data in the study and take responsibility for the integrity of the data and accuracy of the data analysis. The manuscript has been read and approved by all the authors.

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Data Sharing Statement

No additional data are available.

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