Chapter

Hyperglycemia- and Hyperlipidemia-Induced Inflammation and Oxidative Stress through Human T Lymphocytes and Human Aortic Endothelial Cells (HAEC)

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Abstract

Approximately 65% of patients with T2DM die as a result of cardiovascular disease with hyperglycemia and hyperlipidemia being important risk factors for cardiovascular diseases. Both T2DM and atherosclerosis are considered to be inflammatory processes Human T-lymphocytes (T-cells) and aortic endothelial cells (HAEC) have been shown to be components of plaque formation in atherosclerosis. T cells and HAEC are unique in that in their naive state they have no insulin receptors responsive to insulin but become activated in vitro hyperglycemia and in vivo hyperglycemic conditions such as diabetic ketoacidosis and non-ketotic hyperglycemic conditions. Our studies show that T-cells and HAEC in the presence of high concentrations of glucose /and or the saturated fatty acid (SFA) palmitic acid become activated and express insulin receptors, reactive oxygen species (ROS), cytokine elevation, and lipid peroxidation in a time and concentration-dependent manner. Whereas, the unsaturated fatty acid α-linoleic, was not able to activate these cells and had a salutary effect on the activation by glucose and palmitic acid. We have demonstrated that unsaturated fatty acids (UFA) may provide a protective mechanism against the prooxidant effects of hyperglycemia and high SFA such as palmitic acid. Therefore, diet alternations may be beneficial for decreasing hyperglycemia and cardiovascular risks. Studies have shown that lifestyle changes of diet and exercise can reduce the risk of developing diabetes by 58%. Hyperglycemia and hyperlipidemia are important risk factors of developing diabetes and cardiovascular disease. Therefore, we studied the effects of a High Protein diet versus a High Carbohydrate diet in obese non-diabetic, prediabetic and diabetic subjects for effects on weight loss, blood sugar, lipid levels, inflammation, and oxidative stress.

Keywords: blood glucose, free fatty acids, insulin sensitivity, inflammation, cardiovascular risk factors, T lymphocytes, human aortic endothelial cells (HAEC), high protein diet (HP), high carbohydrate diet (HC)

1. Introduction

Greater than 65% of patients with Type 2 Diabetes Mellitus (T2DM) die as a result of cardiovascular disease with hyperglycemia and hyperlipidemia being important risk factors for cardiovascular diseases [1]. Studies have shown that glucose control results in reduction in risk of microvascular complications in diabetes mellitus [2]. The relationship between hyperglycemia and macrovascular complications, however, is not as evident even though subjects with T2DM are two to four times more prone to develop cardiovascular pathology than nondiabetic subjects [3, 4]. Hyperglycemia is a crucial factor contributing to vascular impairment in diabetes mellitus, obesity, and metabolic syndrome [2, 5–7]. Evidence indicates that in hyperglycemic conditions a major contributor to development of large vessel pathology is the endothelial dysfunction [8–10]. Studies have shown that hyperglycemia impairs insulin induced vasodilatory action by decreasing endothelial cells ability to activate nitric oxide synthase-nitric oxide pathway [11]. In hyperglycemic crisis of Diabetic Ketoacidosis (DKA) or hyperglycemic nonketotic state, where both hyperglycemia and high fatty acids are elevated, levels of proinflammatory cytokines and oxidative stress are stimulated [12].

Both T2DM and atherosclerosis are considered to be inflammatory processes [13]. Human T-lymphocytes (T-cells) have been shown to be components of plaque formation along with endothelial cells in atherosclerosis [13]. Human T-lymphocytes have unique properties in that in their naïve state they are insulin unresponsive but upon exposure to high glucose levels they become activated and develop insulin receptors (INR) with emergence of insulin degrading enzyme and insulin responsive glucose uptake [14–19]. Our studies have shown that reactive oxygen species (ROS) along with proinflammatory cytokines such as: Tumor Necrosis Factor α (TNF-α), Interleukin-6 (IL-6), Interleukin-1β (IL-1β) and Interleukin-8 (IL-8); cardiovascular risk markers such as: PAI-1, CRP and Free Fatty Acids (FFA); and counterregulatory hormones such as: IGF-1 and cortisol were all elevated two to three fold above normal with hyperglycemia and returned to normal levels after resolution of hyperglycemia with treatment with insulin and hydration [12]. Our studies have also shown in vitro effects of hyperglycemia (15 and 30 mM) on activation of T-lymphocytes by emergence of IGF-1, INR and Vitamin D receptors and increased levels of ROS and lipid peroxidation [15–18, 20].

Human aortic endothelial cells (HAEC) are also unique in that in their native unstimulated state of glucose of 5 mM they are insulin non-responsive, with GLUT1 as the major glucose transporting protein [21–23]; however, in the presence of high glucose concentrations in a dose dependent manner such as 15 or 30 mM, develop INR and GLUT 4 with glucose uptake [24]. Additionally, high glucose promotes endothelial dysfunction via excessive intracellular glucose accumulation and oxidative stress, leading to increased production proinflammatory cytokines, ROS with induction of the aldose reductase pathway and formation of advanced glycation end products (AGEs) as well as enhanced signaling of protein kinase C (PKC) and mitogen-activated protein kinases (MAPK) [8, 25]. The ROS causes acceleration of cellular growth and promotes synthesis and secretion of proinflammatory cytokines IL-6 and IL-8 which are implicated in the pathogenesis of atherosclerosis [24, 26, 27].

2. Inflammation and oxidative stress effects of glucose and fatty acids on human T-lymphocytes and HAEC

Since both hyperglycemia and hyperlipidemia are important contributing risk for cardiovascular events in diabetes and deleterious effects of hyperglycemia have

been well documented in vivo and in vitro in both T-lymphocytes and HAEC [12, 15, 17, 19, 20, 28–30], we studied the effects of various fatty acids on these cells. The saturated fatty acid palmitic acid, the major fatty acid in plasma, and the monounsaturated, oleic (C18:1), polyunsaturated fatty acids, linoleic (C18:2), omega 3 fatty acid α-linolenic (C18:3), and arachidonic (C20:4) acids were used in 1, 100 and 300 uM concentrations during incubations for 0, 24, 48, 72 and 96 hours. These cells were incubated alone or with glucose concentrations of 5 mM, 15 mM and 30 mM or with a combination with linolenic acid. Unsaturated fatty acids such as linoleic, oleic, linolenic, and arachidonic acids were not able to activate these cells. These studies showed that palmitic acid, but not oleic, linoleic, linolenic or arachidonic acids, exhibited dose and time dependent responses of deleterious effects of palmitic acid by demonstrating increased levels of ROS and lipid peroxidation (malondialdehyde- MDA), emergence of receptors for insulin, IGF-1, IL-2, GLUT 4, inflammatory and proinflammatory effects detected by the emergence of interleukin (IL) cytokines IL-1B, IL-6, IL-8, IL-2, IL-10, and TNFα. and CD69 in T lymphocytes [31]. There was also increased expression of E-selectin, GLUT 4, INR, ROS, lipid peroxidation, inflammatory and proinflammatory effects detected by the emergence of interleukin (IL) cytokines IL-1B, IL-6, IL-8, IL-2, IL-10, and TNFα in the HAEC incubated with palmitic acid and/or glucose. **Table 1** shows the effects of the glucose, palmitic and linolenic incubated alone or in combination with the T lymphocytes. The omega 3 fatty acid linolenic acid had a repressive effect on the deleterious effects of the high glucose and palmitic acid. **Table 2** shows the effects of the glucose, palmitic and linolenic incubated alone or in combination with the HAEC. The omega 3 fatty acid linolenic acid had a repressive effect on the deleterious effects of the high glucose and palmitic acid in the HAEC as well.

Studies have shown that dietary SFA can induce insulin resistance [32–35]. We hypothesized that unsaturated fatty acids (UFA) may provide a protective mechanism against the prooxidant effects of hyperglycemia and high SFA such as palmitic acid. Our studies have also shown that SFA palmitic acid induces expression of GPR40 and FATCD/36 involved in inflammation and the unsaturated fatty acid linolenic acid can reverse the production of these receptors in aortic endothelial cells [36].

Thus, linolenic acid may serve as a protective mechanism against the deleterious effects of high glucose and palmitate in human T-cells and HAEC and reduce the inflammatory process observed with high blood glucose and high saturated fatty acid foods as observed in diabetes, prediabetes, cardiovascular disease and other health conditions.

3. High protein and high carbohydrate diets effects on remission of prediabetes, inflammation, oxidative stress and cardiovascular risk factors

Lifestyle changes of diet and exercise can reduce the risk of developing diabetes by 58% [37]; whereas, pharmaceutical intervention reduces the risk by 32% (metformin) [37] and 72% (pioglitazone) [38]. Hyperglycemia and hyperlipidemia are important risk factors of developing diabetes and cardiovascular disease; however, no diet had been established for reducing the hyperglycemia and hyperlipidemia. Although numerous diets have been recommended for T2DM and non-diabetics [39–43], and suggested advantages of low-carbohydrate [44, 45] or high protein [46, 47] diets, there had been no consensus on a specific weight loss diet to manage blood glucose in T2DM and converting from T2DM to normal glucose tolerance. We studied the effects of macronutrients in a High Protein (HP) (30% Kcal protein,

	Glucose (G)			Palmitic acid (P)			Linolenic acid (L)			G (15 mM) + P (100 μM)	G (15 mM) + L (100 μM)	G (15 mM) + P (100 μM) + L (100 μM)
	5 mM	15 mM	30 mM	5 μM	100 μM	300 μM	5 μM	100 μM	300 μM			
Activation markers												
CD69 (%)	<1	11 ± .7*	26 ± 3*	<1	2 ± 1*	3 ± .8*	<1	<1	<1	14 ± 2†	4 ± .6†	6 ± 1.7Δ
INSR (%)	0	5 ± 1*	12 ± 2*	0	8 ± 2*	14 ± 2*	0	0	0	12 ± 2†	2 ± .6†	5 ± 2Δ
CD25 (%)	0	6 ± 1*	17 ± 3*	0	10 ± 2*	18 ± 3*	0	0	0	15 ± 3†	3 ± .5†	6.5 ± .4Δ
Oxidative stress												
DCF (%)	0	7 ± 1.2*	23 ± 2*	1 ± .3	14 ± 2*	30 ± 3*	0	0	0	21 ± 3†	4 ± .5†	8 ± 2Δ
TBA (MDA)	0	6 ± 2*	19 ± 4*	.8 ± .1	10 ± 2*	22 ± 3*	0	0	0	15 ± 2†	3 + 1†	9 ± 1Δ
Inflammation												
TNFα (pg/ml)	7 ± 1	36 ± 2*	185 ± 4*	7 ± 1	71 ± 3*	98 ± 3*	7 ± 2	8 ± 3	7 ± 2	94 ± 4†	15 ± 2†	49 ± 3Δ
IL-1β (pg/ml)	3 ± 1	18 ± *	51 ± 2*	3 ± 1	21 ± 2*	34 ± 2*	3 ± 1	4 ± 1	3 ± 1	29 ± 3†	11 ± 1†	17 ± 2Δ
IL-6 (pg/ml)	6 ± 1	43 ± 2*	171 ± 3*	6 ± 2	58 ± 3*	87 ± 3*	6 ± 2	7 ± 2	6 ± 2	128 ± 4†	27 ± 3†	71 ± 3Δ
IL-8 (pg/ml)	12 ± 2	184 ± 3*	215 ± 5*	10 ± 2	78 ± 3*	102 ± 4*	9 ± 2	9 ± 2	9 ± 2	235 ± 6†	83 ± 3†	118 ± 3Δ
IL-2 (pg/ml)	23 ± 2	69 ± 3*	172 ± 4*	23 ± 3	87 ± 4*	119 ± 3*	23 ± 2	24 ± 3	23 ± 2	153 ± 4†	36 ± 3†	89 ± 2Δ
IL-10 (pg/ml)	9 ± 1	20 ± 2*	69 ± 3*	9 ± 2	34 ± 3*	58 ± 3*	9 ± 2	9 ± 2	9 ± 2	47 ± 3†	12 ± 1†	29 ± 3Δ
Inflammation inhibition with NFkBi												
TNFα + NFkBi	6 ± 1	6 ± 1	6 ± 2	5 ± 1	5 ± 2	6 ± 2	5 ± 1	5 ± 1	5 ± 1	7 ± 2	7 ± 2	7 ± 3

Values indicate mean ± SE. Measured at 72 hours.
**P < 0.05 from baseline.*
†p < 0.05 from glucose (15 mM) or palmitate (100 uM).
Δp < 0.05 from glucose (15 mM) + palmitate (100 uM).

Table 1.
Effect of glucose, palmitic acid, and α-linolenic acid on activation of human T cells, proinflammatory cytokines and IL-10 and oxidative stress (DCF and MDA) at 72 hrs of incubation.

	Glucose (G)			Palmitic Acid (P)			Linolenic Acid (L)			G (15 mM) + P (100 μM)	G (15 mM) + L (100 μM)	G (15 mM) + P (100 μM) + L (100 μM)
	5 mM	15 mM	30 mM	5 μM	100 μM	300 μM	5 μM	100 μM	300 μM			
E-Selectin%	<1	24 ± 4*	39 ± 3†	<1	18 ± 3*	42 ± 4†	<1	<1	<1	39 ± 7†	11 ± 3†	15 ± 4Δ
INSR (%)	<1	25 ± 3*	37 ± 3†	<1	8 ± 2*	22 ± 3†	<1	<1	<1	30 ± 3†	8 ± 2†	12 ± 2Δ
IGF-IR %	<1	8 ± 2*	20 ± 2†	<1	10 ± 3*	24 ± 3†	<1	<1	<1	16 ± 3†	4 ± 1†	5 ± 2Δ
GLUT 4%	<1	17 ± 3*	34 ± 3†	<1	3 ± 1*	8 ± 2†	<1	<1	<1	22 ± 3†	6 ± 2†	7 ± 2Δ
GLUT 1%	3 ± 1	19 ± 3*	30 ± 3†	3 ± 1	6 ± 2*	13 ± 3†	3 ± 1	3 ± 2	3 ± 2	21 ± 3†	8 ± 2†	9 ± 2Δ
IRS-1%	0	15 ± 3*	34 ± 3†	0	7 ± 2*	11 ± 2†	0	0	0	17 ± 3†	6 ± 2†	8 ± 2Δ
Oxidative stress												
DCF (%)	0	9 ± 1*	26 ± 2†	0	14 ± 2*	34 ± 4†	0	0	0	22 ± 3†	4 ± .5†	7 ± 2Δ
MDA (μM)	0	7 ± 2*	20 ± 4†	0	12 ± 2*	29 ± 4†	0	0	0	17 ± 2†	3 + 1†	8 ± 1Δ
Inflammatory markers												
TNFα (pg/ml)	18 ± 1	39 ± 2*	172 ± 4†^	18 ± 2	23 ± 4*	137 ± 5†	18 ± 2	18 ± 3	19 ± 2	94 ± 4†	15 ± 2†	49 ± 3Δ
IL-1β (pg/ml)	3 ± 1	18 ± 2*	51 ± 2†	2 ± 1	7 ± 2*	10 ± 2†	2 ± 1	3 ± 1	3 ± 1	29 ± 3†	11 ± 1†	17 ± 2Δ
IL-6 (pg/ml)	21 ± 3	36 ± 3*	53 ± 4†	19 ± 3	31 ± 4*	66 ± 4†	17 ± 2	18 ± 3	18 ± 2	128 ± 4†	27 ± 3†	71 ± 3Δ
IL-8 (pg/ml)	171 ± 1	384 ± 6*	915 ± 7†	170±	286 ± 3*	759 ± 9†	167 ± 7	169 ± 9	171 ± 8	335 ± 6†	203 ± 3†	218 ± 3Δ
IL-2 (pg/ml)	0	0	0	0	0	0	0	0	0	0	0	0
IL-10 (pg/ml)	2 ± 1	2 ± 1	2 ± 1	2 ± 1	3 ± 1	3 ± 1	2 ± 1	2 ± 1	2 ± 1	2 ± 1	2 ± 1	2 ± 1

Values indicate mean ± SE. Measured at 72 hours.
**P < 0.05 from baseline.*
†p < 0.05 from glucose (15 mM) or palmitate (100 uM).
Δp < 0.05 from glucose (15 mM) + palmitate (100 uM).

Table 2.
Effect of hyperglycemia (glucose), hyperlipidemia (palmitic acid) on activation of human aortic endothelial cells, inflammation (proinflammatory cytokines and IL-10) and oxidative stress (DCF and MDA) and the salutary effect of α-linolenic acid.

	HP (n = 12)			HC (n = 12)			
Parameters	**Baseline**	**6 months**	**p***	**Baseline**	**6 months**	**p***	**p****
Age	35.9 ± 2.1			35.4 ± 2.0			0.8981
Ethnicity AA/C	7/5			10/2			0.3701†
BMI (kg/m^2)	41.3 ± 1.8	37.3 ± 1.9	0.0005	37.0 ± 1.5	33.5 ± 1.4	0.0005	0.5512
% Weight loss		9.8 ± 1.4	0.0005		9.3 ± 1.6	0.0005	0.9323
HOMA IR	4.0 ± 0.8	1.4 ± 0.2	0.0005	3.7 ± 0.4	2.3 ± 0.3	0.0005	0.0033
ISI (Matsuda Index)	2.7 ± 0.5	6.7 ± 0.5	0.0005	2.6 ± 0.3	3.5 ± 0.4	0.0005	<0.0001
Beta cell function	4.4 ± 0.4	11.8 ± 2.5	0.0005	4.2 ± 0.4	6.3 ± 0.6	0.0005	<0.0001
Cardiovascular risk factors							
TG (mg/dl)	107 ± 10	81 ± 2.7	0.0005	102 ± 5.7	94 ± 3.8	0.0117	0.0907
Free fatty acids (mM)	0.57 ± 0.03	0.45 ± 0.03	0.0010	0.56 ± 0.04	0.73 ± 0.07	0.0342	0.0002
hCRP (mg/L)	5.9 ± 0.2	3.8 ± 0.4	0.0005	5.8 ± 0.2	5.0 ± 0.2	0.0005	0.0003
E-selectin (ng/ml)	42.6 ± 1.5	34.0 ± 1.3	0.0005	43.4 ± 1.3	39.7 ± 1.1	0.0005	0.0007
BP (SBP/DBP)	129/ 83 ± 1.5/1.3	119/ 74 ± 1.1/1.3	0.0005/ 0.0005	128/ 82 ± 1.7/1.4	120/ 75 ± 1.7/1.0	0.0005/ 0.0005	0.1029/ 0.2579
Inflammation							
TNFα (pg/ml)	5.9 ± 1.3	4.1 ± 0.2	0.0005	6.0 ± 0.2	5.1 ± 0.1	0.0005	<0.0001
IL-6 (pg/ml)	6.2 ± 0.2	4.9 ± 0.2	0.0005	5.8 ± 0.2	5.4 ± 0.1	0.0005	<0.0001
Oxidative stress							
DCF (μM)	3.2 ± 0.1	2.4 ± 0.1	0.0005	3.2 ± 0.1	2.9 ± 0.1	0.0005	<0.0001
MDA (μM)	1.1 ± 0.06	0.7 ± 0.05	0.0005	1.1 ± 0.05	0.9 ± 0.05	0.0005	0.0004
% Compliance		94% ±1.5%			91% ± 4.8%		0.3979

Significant level for multiple comparison is set at P = 0.01.
**Indicates Wilcoxon Signed Rank Test.*
***Indicates Wilcoxon Rank Sum Test for 6 months HP vs. HC.*
†Fisher's exact test.
AA/C, African American/Caucasian; BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; hsCRP, high sensitivity C-reactive protein; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; DCF, dichlorofluorescein; MDA, malondialdehyde.

Table 3.
Effect of high protein or high carbohydrate diets on weight loss, insulin sensitivity, Beta cell function, markers of cardiovascular risk factors, inflammation and oxidative stress in obese premenopausal, non-diabetic women.

40% Kcal carbohydrate (CHO) and 30% Kcal fat) diet versus a High Carbohydrate (HC) (15% Kcal protein, 55% Kcal CHO and 30% Kcal fat) diet in obese, normal glucose tolerance premenopausal women [48] (see **Table 3**) and in obese prediabetic (Impaired Glucose Tolerant (IGT)) women and men [49] (see **Table 4**) in randomized, controlled clinical trials. In both studies all food was provided with weekly food pick up with daily menus and weight checks for 6 months. Although our study of IGT subjects [49] and obese, non-diabetic, women [48] had significant

	HP (n = 12)			HC (n = 12)			
Parameters	**Baseline**	**6 months**	**p***	**Baseline**	**6 months**	**p***	**p****
% Remission		100			33.3		0.001
Age	43.1 ± 1.3			41.1 ± 1.7			0.96
Ethnicity AA/C	10/2			9/3			
Female/male	9/3			10/2			
% Weight loss		9.8 ± 1.4	<0.001		11.3 ± 1.8	<0.001	0.692
BMI (kg/m^2)	40.5 ± 1.8	37.3 ± 1.9	<0.001	37.4 ± 1.7	33.8 ± 1.6	0.002	0.391
% LeanBMchange		2.6 ± 0.4	0.002		−3.0 ± 1.1	0.005	0.001
% Fat BM change		−2.5 ± 0.4	0.006		−3.5 ± 0.9	0.007	0.04
Insulin sensitivity							
HbA1c %	6.0 ± 0.015	5.46 ± 0.12	.0005	5.93 ± 0.12	5.73 ± 0.17	0.005	<0.0001
HOMA IR	4.79 ± 0.71	1.58 ± 0.38	0.0005	4.74 ± 0.72	3.34 ± 0.78	0.005	<0.0001
ISI (Matsuda index)	2.3 ± 0.3	6.5 ± 1.1	0.0005	2.3 ± 0.3	3.2 ± 0.4	0.005	0.003
Cardiovascular risk factors							
BP (SBP/DBP)	130/81 ± 3/2	116/72 ± 2/2	0.01/0.01	126/81 ± 3/2	118/74 ± 3/3	0.01/0.01	0.73/0.77
TG (mg/dl)	106.9 ± 10	69.4 ± 6.7	0.001	110.1 ± 11	98.7 ± 9.1	0.002	0.04
LDL (mg/dl)	105.9 ± 4.4	82.4 ± 3.4	0.0005	106.2 ± 5.6	101.9 ± 6.2	0.096	0.037
Cholesterol (mg/dl)	165.3 ± 5.7	151.8 ± 5.3	0.0005	167.9 ± 6.1	161.7 ± 6.3	0.02	0.42
HDL (mg/dl)	44.9 ± 1.7	46.3 ± 1.4	0.10	45.8 ± 2.6	46.2 ± 2.5	0.69	0.85
FFA (mM)	0.74 ± 0.05	0.46 ± 0.04	0.0010	0.74 ± 0.04	0.72 ± 0.05	0.064	0.0001
hCRP (mg/L)	9.1 ± 0.4	4.0 ± 0.3	0.0001	8.8 ± 0.3	6.4 ± 0.2	0.005	0.0003
E-Selectin (ng/ml)	53.7 ± 1.5	35.0 ± 1.1	0.0005	53.4 ± 1.6	44.6 ± 1.7	0.005	0.0005
Proinflammatory cytokines							
TNF-α (pg/ml)	12.8 ± 0.4	3.8 ± 0.2	0.0005	12.5 ± 0.4	9.6 ± 0.3	0.005	<0.0001
IL-6 (pg/ml)	8.57 ± 0.34	4.55 ± 0.13	0.0005	8.43 ± 0.22	6.8 ± 0.11	0.0005	<0.0001
Oxidative stress							
DCF (μM)	3.9 ± 0.2	2.5 ± 0.3	0.0004	4.0 ± 0.1	3.2 ± 0.2	0.01	<0.0001
MDA (μM)	1.5 ± 0.07	0.6 ± 0.04	0.0008	1.5 ± 0.08	1.2 ± 0.04	0.02	0.0004

**Indicates Wilcoxon Signed Rank Test for comparison of Baseline to 6 months.*
***Indicates Wilcoxon Rank Sum Test for 6 months comparison of HP vs. HC.*

Table 4.
Effect of HP or HC diets on remission of prediabetes, weight loss, glucose, insulin sensitivity, cardiovascular risk factors, proinflammatory cytokines and oxidative stress.

weight loss (9–10%) on the HP or HC diets, in both studies the HP diet provided greater improvement in insulin sensitivity, reduced CVR factors, decreased inflammation and inflammatory cytokines, decreased oxidative stress, decreased lipid peroxidation, adipokines markers and increased incretin responses [50, 51]. The HP

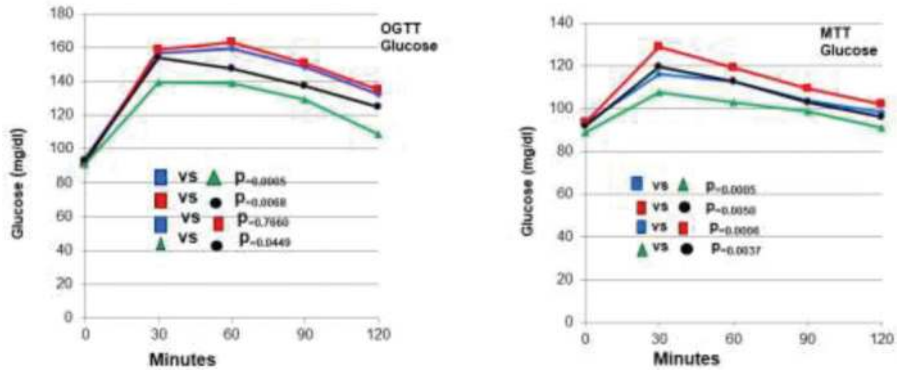

Figure 1.
Obese premenopausal non-diabetic women mean glucose values for the OGTT and MTT from 0 to 120 minutes for the ■ baseline-HP, ■ baseline-HC, ▲ 6 months-HP, and ● 6 months-HC diets for the 12 HP subjects and the 12 HC subjects. P values are the significance of area under the curve for glucose for the OGTT and MTT comparing baseline-HP with 6 month-HP, baseline-HC with 6 month-HC, baseline-HP with baseline-HC, and 6 month-HP with 6 month-HC diets.

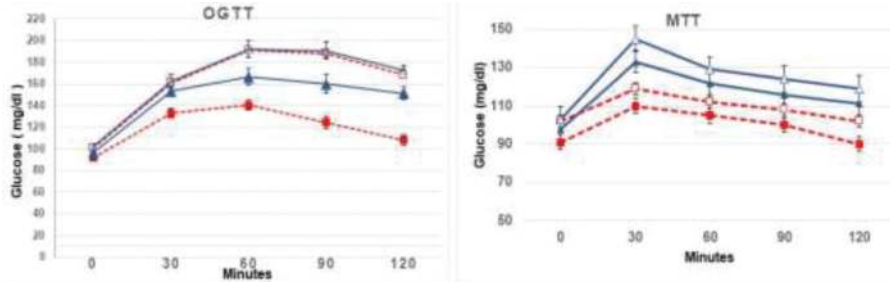

Figure 2.
This figure shows the mean ± SD of glucose for the 2 hour OGTT and MTT for the 12 HP diet subjects and the 12 HC diet subjects. The symbols represent the following: □ HP diet baseline (HP-Bl) and ■ HP diet at 6 months (HP-6 m) dotted lines. △ HC diet baseline (HC-Bl) and ▲ HC diet at 6 months (HC-6 m) solid lines. P values for the glucose AUC for the OGTT are: HP-Bl vs. HP-6 m = 0.0005; HC-Bl vs. HC-6 m = 0.005; HP-Bl vs. HC-Bl = 0.8; HP-6 m vs. HC-6 m = 0.0001. P values for the glucose AUC for the MTT are: HP-Bl vs. HP-6 m = 0.0005; HC-Bl vs. HC-6 m = 0.005; HP-Bl vs. HC-Bl = 0.01, HP-6 m vs. HC-6 m = 0.0001. AUC, area under the curve; HP, High Protein diet; HC, High Carbohydrate diet.

diet caused a smaller increase in blood glucose with the MTT compared to the HC diet MTT in both obese, normal glucose tolerance premenopausal women (see **Figure 1**) and in obese prediabetic women and men (see **Figure 2**), thereby, reducing the hyperglycemia compared to that observed with a HC meal. Lipids are considered a primary risk factor for CV disease and dietary composition can affect the lipid profile and its metabolism. Our studies showed greater decrease in triglycerides in the HP diet demonstrating that increasing the protein in the diet may alter the lipid profile in a beneficial way.

Also, the HP diet subjects had an increase in percent body muscle mass and decrease in percent fat mass at 6 months, whereas, the HC diet subjects had a decrease in percent lean and fat mass. Additionally, the HP group had an increase in their resting metabolic rate (RMR) at 6 months, and an increase in the FGF21 which may be indicative of browning of fat [52, 53]. Epigenetic DNA methylation data showed a change in the methylation with remission of prediabetes in the areas of metabolism, cancer and heart related genes [54, 55].

It has been shown that protein intake by itself induces insulin release and is different in diabetic and non-diabetic individuals [56]. Our study showed a lower insulin response to the HP than the HC diet in both studies. This suggests that HP

diets may help preserve the Beta cells by increasing sensitivity and decreasing insulin load per meal. Our studies on the incretin response to HP and HC diets showed an increased release in GLP-1 and GIP with the HP diet compared to the HC diet [57]. Treatment of T2DM subjects with GLP-1 receptor agonists and DPP-4 inhibitors have been found to improve insulin sensitivity [58, 59]. This suggests that our HP diet may be beneficial in treating T2DM and have cardiovascular benefits.

This prompted us to study the effect of the HP and HC diets on remission of T2DM. Our current randomized clinical trial of Remission of Type 2 Diabetes to normal glucose tolerance (NGT) comparing a HP diet to a HC diet has demonstrated a 100% remission of Type 2 Diabetic subjects to normal glucose tolerance who were diagnosed with the past 2 years and were randomized to the HP diet for 6 months.

However, the HC diet was not very effective with only one subject having remission to normal glucose tolerance. T2D adults aged 20–65 years were randomized to a HP or HC diet for 6 months with all food provided. Caloric need for weight loss was determined by Resting Metabolic Rate (RMR) -500 calories/day. Oral Glucose Tolerance Tests (OGTT) were performed at Baseline and 6 months to determine T2D/NGT status. A Baseline glucose ≥126 mg/dl and HbA1c ≥ 6.5% and 2 hr. glucose >199 mg/dl was considered T2D and remission was a baseline glucose <126 mg/ml and HbA1c < 6.5% and 2 hr. glucose <140 mg/dl. DXA was done at baseline and 6 months to determine bone, lean and fat mass. Food pick up and menus were provided weekly for 6 months along with and weekly weight checks. The T2D subjects on HP diet had 100% remission to NGT while the HC diet subjects had 33% remission. Both diet groups had significant weight loss (HP =15.4 ± 5 lb., HC = 19.9 ± 5 lb), improvement in insulin sensitivity determined by HOMA IR [HP (BL 5.3 ± 0.29; 6 months 2.1 ± 0.13)], [HC (BL 5.2 ± 0.27; 6 months 4.4 ± 0.26)] and decrease in HbA1c [HP (BL 7.7 ± .05; 6 mo 5.6 ± .02)], [HC (BL 7.8 ± .04; 6 mo 6.6 ± .06)] and blood pressure improvement S/D [HP (BL 129/85, 6 months 117/78)], [HC (BL 130/85; 6 months 117/78)]. The HP group had a 2.7% ± 0.4% increase in lean body mass and 2.9 ± 0.4% decrease in fat mass while the HC group had a 1.9% ± 0.3 and 3.3 ± 0.9% decrease in lean and fat mass, respectively. The HP diet provided greater improvement in insulin sensitivity, reduced CVR factors, decreased inflammation and inflammatory cytokines, decreased oxidative stress, decreased lipid peroxidation, adipokines markers and increased incretin responses than the HC diet. Both diets resulted in weight loss, improvement in glucose tolerance and insulin sensitivity but only the HP diet produced 100% remission of T2D to NGT [60].

4. Discussion

High glucose levels initiates inflammatory markers, ROS and hyperglycemia induced pathways in both T lymphocytes and HAEC. Hyperglycemia induced pathways of endothelial damage can activate ERK1/2MAPK cascade via PKC or Advanced Glycation End (AGE) products [4, 25]. High glucose can induce formation of AGE products which are proteins or lipids which become glycated due to exposure to glucose. These AGE products are implicated in aging and many degenerative diseases such as diabetes, atherosclerosis, kidney disease and possibly Alzheimer's disease [61–67]. The glycation of various protein and lipids causes improper function of these molecules, for example, inactivation of anti-aging genes critical to prevent hyperglycemia and hyperlipidemia induced inflammation [68, 69]. We have shown that with remission of prediabetes and better glucose control numerous genes have changes in DNA methylation affecting gene

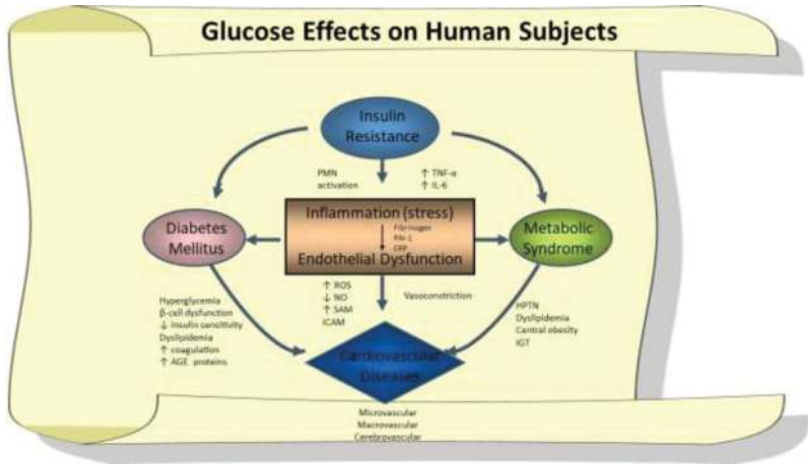

Figure 3.
This figure shows the relationship and effects of high glucose and lipids (dyslipidemia) on inflammation, cardiovascular disease, insulin resistance, diabetes mellitus and metabolic syndrome.

expression and translation of which any of these genes are involved in insulin signaling, cardiovascular disease and inflammation [55]. The effects of hyperglycemia and hyperlipidemia we have shown on the T-lymphocytes will affect the immune system and its function as well as the endothelial cells involved in cardiovascular function. The decrease in inflammation with the decrease in blood glucose and lipids in subjects with the HP diet correlates with the effects of glucose and fatty acids on inflammation and oxidative stress we observed in the in vitro studies of the T-lymphocytes and aortic endothelial cells. This demonstrates the effects of the high glucose and lipids on cells and the whole body. Thus, indicating the importance of good glucose control in diabetic subjects as well as prediabetic and normal subjects to prevent the inflammatory response. **Figure 3** is a diagram of the effects of high glucose on cells and tissues which can be controlled with normal glucose and free fatty acids.

Our previous studies of subjects with Adult Respiratory Distress Syndrome (ARDS) showed the high levels of inflammatory markers. [70, 71]. Reports from the CDC show the deleterious effects COVID-19 has on diabetic subjects indicating that the combined inflammation of the virus plus the high inflammatory markers in diabetic subjects is very detrimental to the subjects. Thus, good glucose control could help decrease the high inflammatory markers observed with the COVID-19.

5. Conclusions

Approximately 65% of patients with T2DM die as a result of cardiovascular disease with hyperglycemia and hyperlipidemia being important risk factors for cardiovascular diseases. Both Type 2 diabetes (T2DM) and atherosclerosis are considered to be inflammatory processes. Human T-lymphocytes (T-cells) and Human Aortic Endothelial cells (HAEC) have been shown to be components of plaque formation in atherosclerosis. T cells and HAEC are unique in that in their naive state they have no insulin receptors responsive to insulin but become activated in the presence of in vitro and in situ hyperglycemic conditions such as diabetic and/or hyperlipidemia of saturated fatty acids (SFA). The unsaturated fatty acid (UFA) α-linolenic acid partially inhibits the activation of T cells induced by either glucose or SFA palmitate alone or in combination. Thus, linolenic acid may serve as a

protective mechanism against the deleterious effects of high glucose and SFAs in human T-cells and reduce the inflammatory process observed with high blood glucose and high saturated fatty acid foods.

A diet which causes a lower blood glucose and lipids after ingestion is beneficial in controlling glucose and lipids as we have shown with the High Protein Diet. The HP diet resulted in 100% remission of pre-diabetes to normal glucose tolerance while the HC diet resulted in only 33% remission. These results show that high efficacy can be achieved with dietary modification if parameters are rigorously controlled and monitored. The HP group had greater improvement in insulin sensitivity, greater reduction in CVR factors, oxidative stress (ROS) and inflammation than the HC diet group. The HP diet prediabetes group percent lean body mass (LM) increased while percent body FM was decreased; whereas, the HC diet group lost both percent LM and FM. Since all subjects were minimally physically active and there was no physical activity modification during the 6 months on the diets, we were able to study the direct effect of the HP versus HC diets. The HC group sustained higher glucose and insulin levels with both the OGTTs and MTTs compared with the HP diet group after 6 months on the diet. The greater insulin response with the HC diet likely equates to greater stress on β cells. The higher sustained glucose elevation with ingestion of glucose or higher glycemic foods as in the HC diet correlates with increased oxidative stress and inflammation in the HC group compared with the HP group. Antioxidant enzymes induced by repeated intake of excess energy in the form of high-fat, HC diets are not sufficient to block oxidative stress and inflammation in healthy human subjects [72]. Thus, the fact that our HP diet had a significantly greater reduction in blood glucose, free fatty acids, ROS and inflammation markers than the HC diet in prediabetes and normal subjects demonstrates the importance of maintaining good glucose control and is of great health importance.

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