SUMMARY& CONCLUSION

Cellular energy metabolism consists of a number of pathways. Mitochondrial respiration and ATP synthesis are two pathways lying at the heart of metabolism. Mitochondrial respiration consists of the oxidation of mitochondrial substrate (NADH) supplied by nutrients which couple the electron transport chain (ETC) to the pumping of proton out across the mitochondrial membrane. The ATP synthase couples the transport of proton across the membrane to the synthesis of ATP inside the mitochondrial matrix. Thus mitochondria convert energy stored in nutrient into ATP/ADP that drives work within the body. However, nothing comes without cost. Molecular oxygen, the final electron acceptor for cytochrome c oxidase, is ultimately reduced to water. However, a small quantity of O_2 may be incompletely reduced as a leakage of single electrons, causes the reduction of O_2 to superoxide ($O_2^{-\cdot}$). Intracellular signaling effectors, including H_2O_2 reflect the balance between the electron leak from the electron transport system, superoxide formation and scavenging of H_2O_2 by endogenous antioxidants in the matrix.

In physiological conditions a homeostatic balance exists between endogenous oxidants, ROS formation and their elimination by endogenous antioxidants. However, under conditions of overnutrition and physical inactivity, typical to those facing metabolic syndrome, the oversupply of substrates generating surplus reducing equivalents could in turn be expected to elevate the redox state and increasing oxidative stress.

We focus in the present work on oxidative stress events leading to individual disease factor appearance in metabolic syndrome patients. Metabolic syndrome patients show altered mitochondrial metabolism as evidenced by more than 100% increase in 8-OHdG (a critical biomarker of oxidative stress) compared to control. Metabolic abnormalities detected in our results defined the presence of abdominal obesity (increased waist circumference), IR along with its associated hyperinsulinemia, elevated TG and fasting glucose, low HDL and high blood pressure.

We obtain also a linear combination of variables, including: waist circumference, hypertension, hyperglycemia, IR, dyslipidemia and oxidative stress markers (8-OHdG, MDA and SOD). Abdominal obesity (increase WC) is consistent with increased oxidative stress markers, representing the trigger for systemic oxidative alterations. Positive correlation between fasting glucose and 8-OHdG suggests a role of hyperglycemia in increase of intracellular glucose metabolism and consequent increase in ROS production.

High plasma oxidative stress markers (8-OHdG& MDA) correlate also positively with elevated TG, LDL and with low HDL. Also, Malondialdehyde (lipid peroxidation index) correlates with low HDL. It is likely that stressors in various forms hit the organism at many different levels (molecular integration between lipid and glucose) and that this adds up to a pathogenic process moving toward insulin resistance obtained in our results. Thus, oxidative stress plays a number of potential mechanisms in the pathophysiology of metabolic syndrome.

Positive correlation between oxidative stress markers and insulin resistance in our metabolic syndrome patients may suggest that several factors that cause insulin resistance have a common pathway in the excessive formation of ROS. Thus, the present work links intracellular metabolic balance to the control of insulin sensitivity. We also place the

etiology of obesity-induced insulin resistance, which is a component of metabolic syndrome in the context of mitochondrial bioenergetics.

Several studies have suggested that increased oxidants by chronic overnutrition coupled with decreased endogenous antioxidant capacity results in oxidative stress. In the present work a significant decrease in total SOD enzyme suggests decreased endogenous mitochondrial capacity in our metabolic syndrome patients.

Accordingly, for many years, interest has focused on strategies that enhance removal of ROS using either antioxidants or drugs that enhance endogenous antioxidant defense. Another object in the present study was to evaluate the prognostic value of oxidative stress markers in a group of metabolic syndrome subjects receiving atorvastatin treatment (a synthetic lipid-lowering agent), and a second group receiving atorvastatin plus vitamin E (as an antioxidant), both groups were followed up for 3 months.

Our results reveal improvements in the quantitative estimation of oxidative stress markers (8-OHdG, MDA and SOD) in both groups. However, this level of supplementation with combined treatment (atorvastatin+ vitamin E) did not alter significantly 8-OHdG, while it decreased significantly MDA and LDL levels when compared with treatment with atorvastatin alone, reflecting the importance of antioxidant vitamins in reducing lipid peroxidation and oxidative stress level.

Therefore, combination therapy that simultaneously addresses multiple mechanisms for the pathogenesis of metabolic disorders is an attractive emerging concept for slowing progression of oxidative stress complications. Combined therapy with statins and antioxidant vitamins demonstrates additive beneficial effects on dyslipidemia and lipid peroxidation when compared with monotherapies in patients with metabolic syndrome and cardiovascular risk factors due to both distinct and interrelated mechanisms. These additive beneficial effects of combined therapies are consistent with laboratory and recent clinical studies. Thus, combination therapy may be an important paradigm for treating and slowing progression of atherosclerosis, coronary heart disease, and co-morbid metabolic disorders characterized by endothelial dysfunction and hyperlipidemia.

We suggest that oxidative stress markers correlate with outcomes in metabolic syndrome patients treated with atorvastatin revealing antioxidant properties of atorvastatin by reducing lipid peroxidation (MDA) and ROS production (8-OHdG). Significant reduction in cholesterol, LDL, TG and obvious increase in HDL in both groups was also obtained.

We can suggest also that the beneficial effect of atorvastatin appear to be greater than that might be expected from changes in lipid levels alone, and as a result of a direct decrease in oxidative stress.

We can suggest that mitochondrial dysfunction leading to increased oxidative stress markers (8-OHdG& MDA) may occur during overnutrition coupled with limited physical activity, and thereby contribute to the genesis and maintenance of the metabolic syndrome.

We suggest a great benefit of dietary intervention for the metabolic syndrome population, i.e. simply reestablishing cellular metabolic balance by limiting caloric intake and/or increasing metabolic demand through increased physical activity. Insulin resistance,

elevated triglycerides and defect of mitochondrial oxidative capacity are all related abnormalities switched-on in response to metabolic stress. We can say also that oxidative stress may be a major determinant factor in the loss of both insulin sensitivity and mitochondrial function associated with overnutrition. Thus oxidative stress plays a number of potential mechanisms in the pathophysiology of metabolic syndrome.

Thus present study suggests that mitochondrial dysfunction leading to oxidative stress as evidenced by increased (8-OHdG& MDA) is a consequence of altered cellular metabolism that develops with nutritional overload. We recommend further testing and exploration of this proposal for the role of mitochondria in the metabolic syndrome which may contribute to our understanding of its origin, maintenance and the development of further   therapy.

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APPENDIX

Table (I): Age (years), body mass index (kg/m^2), waist circumference (cm), waist-hip ratio (WHR), systolic and diastolic blood pressure (B.P) of normal control subjects.

Case number	Age (years)	BMI (kg/m^2)	WC (cm)	WHR	Systolic BP (mmHg)	Diastolic BP (mmHg)
1	40	24.51	81	0.85	120	80
2	45	22.66	93	0.86	130	80
3	44	24.15	86	0.868	120	75
4	40	25.56	89	0.89	110	70
5	43	24.49	93	0.86	120	80
6	46	25.10	96	0.9142	130	85
7	44	25.73	90	0.86	130	80
8	52	25.62	90	0.9	125	85
9	53	25.90	95	0.87	120	80
10	49	25.85	92	0.89	120	85
11	56	27.46	94	0.903	130	90
12	49	25.06	87	0.805	120	80
13	47	24.86	87	0.887	130	85
14	47	23.74	86	0.886	120	80
15	49	23.84	85	0.885	135	80
16	48	26.37	93	0.89	120	80
17	43	25.85	89	0.88	120	80
18	46	24.30	88	0.846	110	75
19	55	24.15	85	0.876	120	85
20	49	25.28	92	0.8679	110	70
Range	40-56	22.66-27.46	81-96	0.81-0.91	110-135	70-90
Mean ±SD	47.25 ±4.45	25.02 ±1.09	89.55±3.94	0.87 ±0.02	122 ±7.14	80.25 ±4.99

Table (II):   Age (years), body mass index (kg/m^2), waist circumference (cm), waist-hip ratio (WHR), systolic and diastolic blood pressure (B.P) of metabolic syndrome patients.

Case number	Age (years)	BMI (kg/m^2)	WC (cm)	WHR	Systolic BP (mmHg)	Diastolic BP (mmHg)
1	57	45.87	127	1.09	170	110
2	56	44.46	124	1.04	160	105
3	49	43.07	122	1.04	150	105
4	49	41.53	122	1.15	150	105
5	49	41.45	121	1.13	140	100
6	54	40.79	117	1.08	140	100
7	47	40.70	121	1.02	140	100
8	43	40.30	119	1.03	140	100
9	45	39.91	115	1.19	140	95
10	44	39.41	119	1.11	135	95
11	43	38.39	121	1.16	135	95
12	43	38.20	117	1.08	135	95
13	47	38.01	119	1.07	130	95
14	41	35.19	114	1.06	130	95
15	40	31.74	107	1.13	130	80
16	56	48.90	130	1.10	180	110
17	55	47.02	125	1.0869	165	110
18	52	45.33	123	1.05	145	105
19	49	43.25	122	1.119	140	100
20	49	42.30	120	1.13	140	100
21	48	41.82	113	1.0089	140	100
22	47	40.96	119	1.09	140	100
23	45	40.48	119	1.0258	140	100
24	45	39.91	118	1.216	140	100
25	44	38.62	116	1.08	140	95
26	43	38.27	115	1.1057	140	95
27	43	37.11	114	1.055	140	95
28	42	35.26	120	1.08	135	95
29	41	33.13	111	1.0277	135	95
30	40	32.69	108	1.1739	130	90
Range	40–57	31.74-48.9	107-130	1.01-1.22	130-180	80-110
Mean ±SD	46.87 ±5	40.14±4.09	118.6±5.13	1.09 ±0.05	142.5±11.87	98.83±6.25

Table (III): Systolic and diastolic blood pressure (B.P) of metabolic syndrome subgroups (IIa)& (IIb) after treatment with either atorvastatin or (atorvastatin + vitamin E).

Case number	MS patients subgroup (IIa)		MS patients subgroup (IIb)	
	Systolic B.P	Diastolic B.P	Systolic B.P	Diastolic B.P
1	170	105	170	110
2	160	105	170	110
3	145	110	140	100
4	155	105	135	100
5	140	100	130	105
6	140	100	135	90
7	150	100	140	100
8	140	100	140	95
9	135	95	140	110
10	135	90	140	100
11	140	95	140	90
12	130	90	145	95
13	130	95	140	100
14	120	90	130	90
15	120	90	120	85
Range	120 – 170	90 – 110	120 – 170	85 – 110
Mean ±SD	140.66 ±13.87	98 ±6.49	141 ±13.25	98.66 ±7.89

Table (IV): Fasting serum glucose (mg/dL), serum insulin (µIU/mL) and homeostasis model assessment-insulin resistance (HOMA-IR) of normal control subjects.

Case number	Fasting glucose (mg/dL)	Serum insulin (µIU/mL)	HOMA-IR
1	95	9	2.11
2	84	8.5	1.76
3	88	9	1.96
4	93	7	1.61
5	85	7.5	1.57
6	82	10	2.02
7	87	8	1.72
8	86	9.2	1.95
9	100	9.8	2.42
10	81	6	1.20
11	79	9.6	1.87
12	84	5	1.04
13	92	8	1.82
14	97	7.4	1.77
15	75	4.4	0.81
16	83	7	1.43
17	86	9	1.91
18	96	7.4	1.75
19	91	8.6	1.93
20	90	9	2.00
Range	75 – 100	4.4 – 10	0.81 – 2.42
Mean ±SD	87.7 ±6.48	7.97 ±1.53	1.73 ±0.378

Table (V): Fasting serum glucose (mg/dL), serum insulin (μIU/mL) and homeostasis model assessment-insulin resistance (HOMA-IR) of metabolic syndrome subgroup (IIa) before and after treatment with atorvastatin.

Case number	Fasting glucose (mg/dL)		Serum insulin (μIU/mL)		HOMA-IR	
	Before treat.	After treat.	Before treat.	After treat.	Before treat.	After treat.
1	240	245	12	11.8	7.11	7.11
2	200	190	14	14.3	6.91	6.7
3	143	152	17	15	6	5.63
4	140	145	17	16.6	5.88	5.94
5	129	138	19	18.6	6.05	6.34
6	128	129	19	22	6	7.01
7	120	114	20	19.6	5.93	5.52
8	118	123	20	23	5.83	6.99
9	114	124	22	23	6.19	7.04
10	113	106	14	21.5	3.91	5.63
11	110	120	24	19	6.52	5.63
12	109	99	24	23.5	6.46	5.74
13	107	101	26	31	6.87	7.73
14	105	110	27	26.5	7	7.2
15	94	97	29	30.5	6.73	7.3
Range	94 – 240	97 – 245	12 – 29	11.8 – 31	3.91 – 7.11	5.52 – 7.73
Mean ±SD	131.33±39.03	132.86±39.44	20.26±5.04	21.06±5.55	6.22±0.78	6.5±0.75

Table (VI): Fasting serum glucose (mg/dL), serum insulin (µIU/mL) and homeostasis model assessment-insulin resistance (HOMA-IR) of metabolic syndrome subgroup (IIb) before and after treatment with atorvastatin + vitamin E.

Case number	Fasting glucose (mg/dL)		Serum insulin (µIU/mL)		HOMA-IR	
	Before treat.	After treat.	Before treat.	After treat.	Before treat.	After treat.
1	160	179	14	16	5.53	7.07
2	150	130	17	17.4	6.3	5.59
3	140	132	18	20	6.22	6.52
4	130	120	19	19.5	6.1	5.76
5	126	133	20	19.6	6.22	6.44
6	121	119	20	20.5	5.98	6.02
7	117	110	21	19.8	6.07	5.38
8	115	109	22	22.5	6.25	6.06
9	113	109	23	23.6	6.42	6.35
10	110	112	17	22.4	4.62	6.21
11	109	102	24	25	6.46	6.3
12	108	103	25	22	6.67	5.6
13	104	119	27	28.6	6.93	8.4
14	95	99	29	28.4	6.8	6.94
15	88	85	31	30	6.74	6.3
Range	88 – 160	85 – 179	14 – 31	16 – 30	4.62 – 6.93	5.38 – 8.4
Mean ±SD	119.06 ±19.59	117.4 ±21.51	21.8 ±4.75	22.35 ±4.13	6.22 ±0.57	6.32 ±0.74

Table (VII): High-density lipoprotein (HDL mg/dL), triglycerides (TG mg/dL), total cholesterol (mg/dL) and low-density lipoprotein (LDL mg/dL) of normal control subjects.

Case number	HDL (mg/dL)	Triglycerides (mg/dL)	T.Cholest (mg/dL)	LDL (mg/dL)
1	40	142	160	91.6
2	49	129	174	99.2
3	65	137	166	73.6
4	61	121	153	67.8
5	52	149	135	53.2
6	36	143	185	120.4
7	60	139	149	61.2
8	44	134	178	107.2
9	39	133	163	97.4
10	44	136	144	72.8
11	48	140	175	99
12	59	135	139	53
13	50	139	145	67.2
14	55	129	159	78.2
15	47	144	164	88.2
16	52	141	155	74.8
17	56	130	190	108
18	62	146	182	90.8
19	54	137	141	59.6
20	47	126	162	89.8
Range	36 – 65	121 – 149	135 – 190	53 – 120.4
Mean ±SD	51 ±8.1	136.5 ±7.05	160.95 ±16.06	82.65 ±19.35

Table (VIII): High-density lipoprotein (HDL mg/dL), triglycerides (TG mg/dL), total cholesterol (mg/dL) and low-density lipoprotein (LDL mg/dL) of metabolic syndrome subgroup (IIa) before treatment with atorvastatin.

Case number	HDL (mg/dL)	Triglycerides (mg/dL)	T.Cholest (mg/dL)	LDL (mg/dL)
1	24	490	333	211
2	30	330	295	199
3	32	420	310	194
4	35	259	290	203.2
5	37	280	300	207
6	36	310	305	207
7	36	250	290	204
8	30	220	260	186
9	30	210	270	198
10	31	230	260	183
11	32	190	250	180
12	33	230	260	181
13	34	210	235	159
14	39	180	240	165
15	46	168	240	160.4
Range	24 – 46	168 – 490	235 – 333	159 – 211
Mean ±SD	33.66 ±5	265.13 ±90.3	275.86 ±29.67	189.17 ±17.41

Table (IX): High-density lipoprotein (HDL mg/dL), triglycerides (TG mg/dL), total cholesterol (mg/dL) and low-density lipoprotein (LDL mg/dL) of metabolic syndrome subgroup (IIa) after treatment with atorvastatin.

Case number	HDL (mg/dL)	Triglycerides (mg/dL)	T.Cholest (mg/dL)	LDL (mg/dL)
1	26	390	233	129.02
2	31	284	207	118.39
3	33	361	217	111.32
4	37	223	203	121.877
5	41	188	198	119.04
6	38	267	214	122.56
7	38	215	203	122.38
8	34	147	172	108.52
9	31	181	189	121.53
10	35	154	172	106.06
11	33	163	175	108.88
12	37	154	172	103.82
13	37	165	163	93.1
14	41	110	161	98.245
15	48	144	168	91.034
Range	26 – 48	110 – 390	161 – 233	91 – 129.02
Mean ±SD	36 ±5.19	209.73 ±82.06	189.8 ±22.51	111.71 ±11.64

Table (X): High-density lipoprotein (HDL mg/dL), triglycerides (TG mg/dL), total cholesterol (mg/dL) and low-density lipoprotein (LDL mg/dL) of metabolic syndrome subgroup (IIb) before treatment with atorvastatin + vitamin E.

Case number	HDL (mg/dL)	Triglycerides (mg/dL)	T.Cholest (mg/dL)	LDL (mg/dL)
1	29	250	280	201
2	30	408	320	208.4
3	33	270	300	213
4	31	230	280	203
5	28	220	285	213
6	28	350	326	228
7	35	300	295	200
8	30	260	280	198
9	30	200	230	160
10	32	210	265	191
11	33	215	240	164
12	34	230	240	160
13	35	200	240	165
14	39	155	260	190
15	44	150	240	166
Range	28 – 44	150 – 408	230 – 326	160 – 228
Mean ±SD	32.73 ±4.33	243.2 ±68.56	272.06 ±30.41	190.69 ±22.3

Table (XI): High-density lipoprotein (HDL mg/dL), triglycerides (TG mg/dL), total cholesterol (mg/dL) and low-density lipoprotein (LDL mg/dL) of metabolic syndrome subgroup (IIb) after treatment with atorvastatin + vitamin E.

Case number	HDL (mg/dL)	Triglycerides (mg/dL)	T.Cholest (mg/dL)	LDL (mg/dL)
1	35.67	175	173.6	102.93
2	36.9	285.6	198.4	104.38
3	40.59	189	186	107.61
4	38.13	161	173.6	103.27
5	34.44	154	176.7	111.46
6	34.44	245	202.12	118.68
7	39.2	201	194.7	115.3
8	36.9	182	173.6	100.3
9	33.6	134	151.8	91.4
10	39.36	147	164.3	95.54
11	40.59	150.5	148.8	78.11
12	41.82	161	148.8	74.78
13	41.6	140	148.8	79.2
14	47.97	108.5	161.2	91.53
15	45.98	129	168	96.22
Range	33.6 – 47.97	108.5 – 285.6	148.8 – 202.12	74.78 – 118.68
Mean ±SD	39.14 ±4.14	170.84 ±45.86	171.36 ±18.03	98.04 ±13.31

Table (XII): Serum 8-hydroxy-2'-deoxyguanosine (8-OHdG ng/mL), malondialdehyde (MDA nmol/mL) and superoxide dismutase (SOD unit/mL) of normal control subjects.

Case number	8-OHdG (ng/mL)	MDA (nmol/mL)	SOD (U/mL)
1	7.7	2	13
2	5.8	2.4	11.1
3	5.4	3	11
4	5.5	2.9	10.9
5	5	3.3	10.6
6	7.5	4.5	7.9
7	5.6	3.5	9.9
8	6.3	4.2	8
9	7.2	4.1	8.3
10	5.3	2.6	9.4
11	6.5	3.9	8.5
12	4.9	3.1	9.2
13	4.8	3.3	9
14	4.7	3.3	8.8
15	4.5	2.9	8.7
16	4.3	2.4	8.6
17	4	3.4	9.3
18	3.1	6	9.6
19	4.1	2.5	9.8
20	1.8	2	10.4
Range	1.8 – 7.7	2 – 6	7.9 – 13
Mean ±SD	5.2 ±1.44	3.26 ±0.94	9.6 ±1.27

Table (XIII): Serum 8-hydroxy-2'-deoxyguanosine (8-OHdG ng/mL), malondialdehyde (MDA nmol/mL) and superoxide dismutase (SOD unit/mL) of metabolic syndrome subgroup (IIa) before and after treatment with atorvastatin.

Case number	8-OHdG (ng/mL)		MDA (nmol/mL)		SOD (U/mL)	
	Before treat.	After treat.	Before treat.	After treat.	Before treat.	After treat.
1	19.4	13.8	12.0	8.4	5.0	6.1
2	18.6	13.3	11.9	7.6	5.0	6.1
3	18.4	12.0	11.3	7.9	5.1	6.3
4	16.9	12.1	11.2	7.8	5.4	6.6
5	15.4	11.0	11.2	7.6	5.6	6.8
6	13.7	9.8	11.2	6.7	5.6	6.8
7	13.2	9.1	11.0	7.7	5.7	7.0
8	13.0	9.3	11.0	6.7	5.7	7.0
9	12.3	8.8	10.9	7.6	5.8	7.1
10	11.8	7.7	10.6	6.5	6.0	7.3
11	11.0	7.9	10.5	7.4	6.1	7.5
12	9.8	7.0	10.2	7.1	6.1	7.5
13	6.4	4.1	10.0	7.0	6.1	7.5
14	5.6	4.0	8.3	5.8	6.6	8.0
15	4.4	4.6	7.7	5.4	6.9	8.4
Range	4.4 – 19.4	3.2 – 13.8	7.7 – 12	5.4 – 8.4	5 – 6.9	6.1 – 8.4
Mean ±SD	12.66 ±4.7	8.87 ±3.31	10.6 ±1.19	7.14 ±0.81	5.78 ±0.54	7.06 ±0.65

Table (XIV): Serum 8-hydroxy-2'-deoxyguanosine (8-OHdG ng/mL), malondialdehyde (MDA nmol/mL) and superoxide dismutase (SOD unit/mL) of metabolic syndrome subgroup (IIb) before and after treatment with atorvastatin + vitamin E.

Case number	8-OHdG (ng/mL)		MDA (nmol/mL)		SOD (U/mL)	
	Before treat.	After treat.	Before treat.	After treat.	Before treat.	After treat.
1	18.87	12.27	12	7.32	4.898	6.24
2	18.38	11.95	11.9	7.26	5.084	6.27
3	17.65	11.37	11.6	7.08	5.27	6.47
4	15.93	10.36	11.3	6.89	5.332	6.54
5	14.22	10.15	11.2	6.40	5.456	6.92
6	13.48	8.76	11	5.94	5.58	6.98
7	13.24	6.35	11	5.97	5.642	7.14
8	12.26	7.97	10.5	5.94	5.766	7.38
9	12.01	7.81	10.5	6.05	5.952	7.46
10	11.52	5.53	10	5.67	6.076	7.62
11	10.54	6.85	10	5.49	6.448	7.91
12	7.60	3.65	9.9	5.42	6.51	8.32
13	6.13	3.98	9.8	5.40	6.758	8.33
14	4.90	3.19	9	5.40	6.82	8.65
15	4.17	4.5	8.9	5.34	8.06	10.32
Range	4.17 – 18.87	2 – 12.27	8.9 – 12	5.34 – 7.32	4.9 – 8.06	6.24 – 10.32
Mean ±SD	12.06 ±4.71	7.47 ±3.34	10.57 ±0.96	6.1 ±0.71	5.97 ±0.829	7.5 ±1.09

التلف التأكسدي للحمض النووي (دي إن إيه) عند المرضى بمتلازمة الأيض

Oxidative DNA damage in patients with metabolic syndrome

Protocol of a thesis submitted	خطة بحث مقدمة إلى
to the Medical Research Institute	معهد البحوث الطبية
University of Alexandria in	جامعة الإسكندرية
partial fulfillment of the	إيفاءا جزئيًا لشروط
requirements of the degree of	الحصول على درجة

M.D. of Applied Medical Chemistry دكتوراه في الكيمياء الطبية التطبيقية

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2009	٢٠٠9

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BACKGROUND

It is estimated that around a quarter of the world's adult population have metabolic syndrome[1] and they are twice as likely to die from and three times as likely to have a heart attack or stroke compared with people without the syndrome.[2]

A person is defined as having the metabolic syndrome if he has central obesity (waist circumference≥94cm for European men and≥80 cm for European women) with ethnicity specific values for other groups plus two of the following four factors:

a) Raised triglycerides level: ≥150mg/dL (1.7mmol/L) or specific treatment for this lipid abnormality.

b) Reduced high density lipoprotein cholesterol<40mg/dL (1.03mmol/L) in males and <50mg/dL (1.29mmol/L) in females or specific treatment for this lipid abnormality.

c) Raised blood pressure: systolic blood pressure≥130 or diastolic blood pressure≥85mmHg or treatment of previously diagnosed hypertension.

d) Raised fasting plasma glucose≥100mg/dL (5.6mmol/L) or previously diagnosed type II diabetes. If above 5.6mmol/L or 100mg/dL, oral glucose tolerance test is strongly recommended but is not necessary to define the presence of this syndrome.[3]

Hypertriglyceridemia, hyperglycemia, and insulin resistance are closely linked to elevated systemic oxidative stress in metabolic syndrome, which is, in turn, reflected in the impairment of HDL antioxidative properties. Dysfunctional HDL subfractions play a central role in the expression of elevated oxidative stress in metabolic syndrome. Because oxidative stress is a key component of endothelial dysfunction, and thus of elevated cardiovascular risk in metabolic syndrome, these results lead us to propose that early treatment of metabolic

syndrome patients, targeted at reduction of oxidative stress and normalization of HDL function, is necessary, even in the absence of diabetes.[4]

Lipid peroxidation is frequently investigated in biomedical research, and malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acids peroxidation in the cells.[5]

The transfer of a single electron to O_2 forms superoxide anion, whereas the transfer of two electrons yields peroxide. Superoxide Dismutase (SOD) catalyzes the conversion of superoxide anion (O_2^-.) to hydrogen peroxide (H_2O_2) and oxygen (O_2), and provides the primary cellular defense against the toxicity of superoxide anion.[6]

The free radicals activity is evaluated by measuring their pathophysiologic products.[7] DNA is probably the most biologically significant target of oxidative attack, and it is widely thought that continuous oxidative damage to DNA is a significant contributor to the age-related development of the major cancers, such as those of the colon, breast, rectum, and prostate.[8]

Recently, it was found that reactive oxygen species, irrespective of their origin, may interact with cellular biomolecules, such as DNA, leading to modification and potentially serious consequences for the cell.[9]

Deoxyguanosine (dG) is one of the constituents of DNA and when it is oxidized, it is altered into 8-hydroxy-2'-deoxyguanosine (8-OH-dG).[10] (8-OH-dG) is a quantitative measurement of the oxidative DNA adducts in various biological samples such as DNA serum, urine etc. Numerous evidences have indicated that urinary (8-OH-dG) not only is a biomarker of DNA damage but might also be a risk factor for cancer, atherosclerosis and diabetes.[11]

During the repair of damaged DNA in vivo by exonucleases, the resulting 8-OH-dG is excreted without further metabolism, and can be measured as a biomarker of oxidative DNA damage.[12]

Statins (hydroxyl-B-methylglutaryl coenzyme-A reductase inhibitors) were developed as cholesterol-lowering agents and found to have antioxidative effects. Various studies have also demonstrated that statins decrease the frequency of coronary heart disease. The antioxidative effect exhibited by statins is considered to be one of the mechanisms behind the anti-atherogenic effect.[13]

Several large-scale clinical trials have assessed the efficacy of atorvastatin in the primary and secondary prevention of cardiovascular events in patients with diabetes mellitus and/or metabolic syndrome.[14]

AIM OF THE WORK

The objective of the present study is the measurement of the serum level of 8-hydroxydeoxyguanosine (8-OHdG), Malondialdehyde (MDA), which reflects the tissue lipid peroxidation level and Superoxide Dismutase (SOD), representing the activity of hepatic antioxidant enzymes, in patients with metabolic syndrome. The effect of Atorvastatin and Vitamin E on these parameters in different groups will be also studied.

SUBJECTS AND METHODS

This study will be performed on a total of 30 obese male patients who fulfilled the definition criteria of metabolic syndrome according to the International Diabetes Federation (IDF) definition, their ages range from 40-60 years.

Patients with metabolic syndrome will be assigned to two groups randomly. One group will be administered 10mg/day of atorvastatin, and the other group 10mg/day of atorvastatin plus 1000mg/day of vitamin E for 3 months.

A group of 20 age-matched healthy non-obese males will be used as a control group.

All patients and controls will be subjected to the following:

A) Complete medical history taking with special emphasis on: age, special habits (smoking, diet, exercise, medications,...), hypertension, diabetes mellitus, dyslipidemia, ischemic heart disease.

B) Thorough clinical examination with special emphasis on: blood pressure, height, weight, waist circumference, hip circumference.
Body mass index will be calculated:

Body mass index (BMI)= weight in Kilograms divided by the square of height in Meters; (obesity BMI>30).

Blood pressure will be measured in a duplicate by mean of a standard sphygmomanometer.

C) Laboratory analysis: blood samples will be collected after an overnight fasting (12-h), serum will be used for the following:

- Fasting and 2 hours post prandial blood glucose by colorimetric method.[15]

- Lipid profile by colorimetric method.[16,17,18,19]

- Fasting insulin concentration by ELISA.

- Estimation of serum (8-OHdG) as a marker of oxidative stress.

- Estimation of serum (MDA) as a marker of oxidative stress.

- Estimation of serum (SOD) as a marker of antioxidant liver enzymes.

The patients with metabolic syndrome will be subjected to all investigations twice, pre and post oral treatment with atorvastatin (group I), and with atorvastatin plus vitamin E (group II) for three months.

In this study, we will use atorvastatin tablets, once/day (Ator 10mg) (EPICO- 10th of Ramadan city, Egypt). Also we will use vitamin E capsules, once/day (E1000) (SEDICO- 6th of October city, Egypt).

Patients with metabolic syndrome will be selected from the outpatients' clinics. Healthy individuals will be recruited consecutively among physicians and co-workers, represent normal-weight, control subjects.

All subjects will give written informed consent to participate in this study, which will be approved by the local ethical committee; in accordance with the Declaration of Helsinki.

Estimation of serum (8-OHdG):[20]

This will be done by Cell Biolabs' Oxidative DNA Damage ELISA Kit. (USA). It is a competitive enzyme immunoassay developed for rapid detection and quantitation of 8-OHdG in urine, serum, or other cell or tissue DNA samples.

Estimation of fasting insulin concentration:[21]

By ELISA kit provided by (Biosource Europe S.A, Nivelles, Belgium).

Estimation of serum (MDA):[22,23]

This will be done by Cayman's TBARS Assay Kit.

Estimation of serum (SOD):[24]

By Cayman Chemical Superoxide Dismutase Assay Kit (USA), using activity method.

ANALYSIS OF RESULTS

The results obtained will be statistically evaluated by comparing them with the data obtained from the corresponding control groups. A t-test will be used for two group comparisons. ANOVA will be used for comparisons of multiple means. P values less than 0.05 will be considered significant.

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الملخص العربي

قدرة الإنسان على استعمال الأكسجين في عملية التنفس أمدته بفائدة استعمال أيض المواد الغذائية (دهون- بروتينات- كربوهيدرات) في إنتاج الطاقة.

تتكون عملية التمثيل الغذائي للطاقة داخل الخلايا من العديد من مسارات التفاعلات الكيميائية، حيث تقع عمليتي التنفس الخلوي وتكوين جزيئات الطاقة واللتان تتمان في الميتوكندريا في صلب عملية الأيض.

عملية التنفس الخلوي في الميتوكندريا تتم بأكسدة مادة (NADH) الممولة من المواد الغذائية عن طريق النقل الإلكتروني، وبالتالي ضخ البروتون خارج غشاء الميتوكندريا، حيث يعمل إنزيم (ATP synthase) الموجود في غشاء الميتوكندريا على الربط بين عبور البروتون وتكوين مركب الطاقة (ATP) داخل جسم الميتوكندريا. وبذلك تكون الميتوكندريا قد حولت الطاقة الكيميائية المختزنة في المواد الغذائية إلى مركب الطاقة (ATP) ليستعملها الجسم.

وعلى الرغم من أهمية هذه العمليات الحيوية فإنه لاشئ يأتي من دون ثمن، فمركب الأكسجين (وهو المستقبل الأخير للإلكترون) يتم في النهاية إختزاله إلى ماء، ولكن جزء يسير حوالي ٥ % من الأكسجين قد لا يتم أكسدته بشكل كامل نتيجة هروب بعض الإلكترونات، مما قد ينتج عنه إختزال غير تام للأكسجين مكونا فوق الأكسيد (O_2^{-}) وبعض المؤكسدات التي تعمل كمؤثرات داخلية في نقل الإشارات بين الخلايا، بما فيها فوق أكسيد الهيدروجين (H_2O_2). حيث يعكس معدل فوق أكسيد الهيدروجين (H_2O_2) التوازن بين كل من التسرب الإلكتروني وتكوُّن فوق الأكسيد وإزالته بواسطة مضادات التأكسد الداخلية الموجودة داخل الميتوكندريا.

في الأحوال الوظيفية الطبيعية للأعضاء يوجد إتزان ما بين عملية تكوين المؤكسدات الداخلية وتكون الشقوق الحرة من جانب، وعملية التخلص منها عن طريق مضادات الأكسدة الداخلية من جانب آخر. ولكن في بعض حالات التغذية المفرطة وعدم استهلاك الطاقة (نتيجة عدم بذل نشاط) وهي الصورة النمطية للمرضى بمتلازمة الأيض، في هذه الحالة تنشأ عن زيادة المواد الغذائية زيادة في المكافئات المختزلة، مما يرفع حالة التأكسد ويزيد من وطأتها.

وقد اختص هذا البحث بدراسة وطأة التأكسد المصاحب للإفراط في تناول الغذاء مع عدم استهلاك الطاقة، مما يؤدي إلى الإصابة ببعض الظواهر المرضية عند المرضى بمتلازمة الأيض. وقد أظهرت النتائج زيادة واضحة في معدلات الأيض داخل الميتوكندريا، حيث ارتفع (8-OHdG) بنسبة تفوق المائة في المائة، وهو أحد الدلالات الهامة لوطأة التأكسد الناتج عن أكسدة وتلف الحمض النووي (DNA)، وكذلك ارتفع مؤشر أكسدة الدهون (المالون داي ألديهيد) (MDA) مقارنة بالمجموعة الضابطة.

كما تم رصد وجود سمنة في منطقة البطن (Waist Circumference)، ومقاومة الإنسولين (Insulin Resistance) مع ما يصاحبها من زيادة في تركيز الإنسولين. كذلك زيادة مستوى الدهون الثلاثية (TG)، والبروتين الدهني منخفض الكثافة (LDL)، وانخفاض البروتين الدهني مرتفع الكثافة (HDL)، وارتفاع ضغط الدم.

وقد حصلنا على ارتباط خطي يسير في نفس الاتجاه بين المتغيرات التالية: محيط البطن، ارتفاع ضغط الدم، ارتفاع السكر في الدم، مقاومة الإنسولين، وكذلك دلالات وطأة التأكسد. حيث تتناسب السمنة البطنية طرديا مع الزيادة في دلالات وطأة التأكسد. كما لوحظ وجود علاقة إيجابية بين مستوى السكر الصائم ووطأة التأكسد مما قد يشير إلى الدور الذي يلعبه ارتفاع السكر في زيادة أيض السكر داخل الخلايا، وبالتالي زيادة إنتاج أنواع المؤكسدات التفاعلية.

كما أشارت نتائج هذا البحث أيضا إلى أن زيادة دلالات وطأة التأكسد في الدم (MDA & 8-OHdG) يتناسب طرديا أيضا مع ارتفاع الدهون الثلاثية (TG)، والبروتين الدهني منخفض الكثافة (LDL)، وعكسيا مع البروتين الدهني مرتفع الكثافة (HDL). كذلك فإن الـ(MDA) (مؤشر أكسدة الدهون) يتناسب مع النقص في البروتين الدهني مرتفع الكثافة (HDL). وبذلك تكون المؤثرات المختلفة التي تصيب الإنسان في مستويات مختلفة نتيجة الخلل الناتج عن التكامل الجزيئي في الأيض بين الدهون والسكريات دافعا إلى حالة مقاومة الإنسولين. لذلك تلعب وطأة التأكسد العديد من الآليات القوية في الخلل الوظيفي للمرضى بمتلازمة الأيض.

وقد أظهرت النتائج وجود علاقة طردية بين دلالات وطأة التأكسد ومقاومة الإنسولين في المرضى المصابين بمتلازمة الأيض مما يشير إلى أن العوامل المتعددة التي تسبب مقاومة الإنسولين تشترك جميعها في زيادة تكوين المؤكسدات التفاعلية. ولذلك فإن هذا البحث يربط بين توازن التمثيل الغذائي داخل الخلايا والتحكم في حساسية الإنسولين.

كما قمنا برصد أسباب مقاومة الإنسولين الناجمة عن السمنة، والتي تعد مكوناً من متلازمة الأيض في سياق تفاعلات الطاقة الحيوية داخل الميتوكوندريا.

كما أوضحت العديد من الدراسات أن زيادة التأكسد بفعل زيادة التغذية مرتبط بالإنخفاض في القدرة الذاتية المضادة للأكسدة (Endogenous Antioxidant)، مما ينجم عنه وطأة التأكسد. لذلك اختص هذا البحث بدراسة السعة الداخلية لمضادات التأكسد داخل الميتوكوندريا عند المرضى بمتلازمة الأيض عن طريق قياس أحد الإنزيمات المضادة للتأكسد وهو إنزيم السوبر أكسيد ديسميوتاز (SOD) حيث لوحظ حدوث انخفاض في هذا الإنزيم.

كما تم التركيز في الأعوام الماضية على بعض الوسائل التي تحفز التخلص من أنواع المؤكسدات التفاعلية إما باستعمال مضادات الأكسدة أو العقاقير التي تحفز دفاعات مضادات الأكسدة الذاتية. لذلك شمل هذا البحث أيضا على محاولة تحفيز التخلص من المؤكسدات التفاعلية في مجموعتين من المرضى بمتلازمة الأيض عن طريق استعمال عقار الأتورفاستاتين (Atorvastatin) في المجموعة الأولى وهو عقار خافض لدهون الدم. وكذلك الجمع بين عقار الأتورفاستاتين وفيتامين هـ المضاد للأكسدة في المجموعة الثانية، حيث تمت متابعة كلتا المجموعتين لمدة ثلاثة أشهر متتالية.

وقد أظهرت نتائجنا حدوث تحسن كمي في دلالات وطأة التأكسد (SOD, MDA, 8-OHdG) في كلتا المجموعتين. ومع ذلك فإن العلاج الثاني في المجموعة الثانية (Atorvastatin + فيتامين هـ) لم يحدث تغيرا ملحوظا في مستوى (8-OHdG)، بينما نجح في تخفيض مستوى كل من الـ(MDA) والبروتين الدهني منخفض الكثافة (LDL) عند مقارنته بالعلاج الأحادي (Atorvastatin) مما يعكس دور الفيتامينات المضادة للأكسدة في خفض وطأة تأكسد الدهون.

ولذلك تظهر بصورة واضحة أهمية العلاج المركب (من أكثر من نوع من مضادات الأكسدة) والذي يستهدف العديد من الآليات المتعددة في علاج مسببات الأمراض الخاصة بالخلل التمثيلي وإبطاء تطور مضاعفات وطأة التأكسد. حيث تفوق العلاج الثاني باستعمال الستاتينز (Statins) مع الفيتامينات مضادات التأكسد في علاج ارتفاع وتأكسد الدهون عند مقارنته بالعلاج الأحادي في المرضى بمتلازمة الأيض نتيجة التأثيرات المحددة والمرتبطة لكل منهم. مما يفتح الباب في استعمال العلاجات المركبة في علاج وتأخير مضاعفات تصلب الشرايين، الناتجة عن ارتفاع الدهون وتلف الأوعية الدموية.

وقد أوضحت النتائج وجود تأثير فعال لعقار الـ (Atorvastatin) كمضاد للأكسدة وليس فقط لمعالجة زيادة دهون الدم، حيث نجح في تقليل وطأة التأكسد لدى المرضى بمتلازمة الأيض، عن طريق تقليل إنتاج أنواع المؤكسدات التفاعلية، مما أفاد في تقليل مؤشر أكسدة الحمض النووي (8-OHdG) وكذلك مؤشر أكسدة الدهون (MDA) إلى جانب خفض الكوليسترول والدهون الثلاثية (TG) والبروتين الدهني منخفض الكثافة (LDL)، وزيادة البروتين الدهني مرتفع الكثافة (HDL).

ويكون من أهم نتائج هذا البحث ظهور التأثير الواضح لعقار الـ (Atorvastatin) في إبطاء تطور مضاعفات وطأة التأكسد، حيث أظهرت النتائج أن له دورا مضادا للأكسدة عن طريق تحسين معدلات الأيض داخل الميتوكندريا إلى جانب دوره في خفض الدهون في الدم. كما خلص هذا البحث إلى أن الخلل الوظيفي للميتوكندريا عند المرضى بمتلازمة الأيض والمؤدي إلى زيادة وطأة التأكسد (المثبتة بزيادة دلالات وطأة التأكسد (MDA& 8-OHdG)) سببه الإفراط في الطعام. لذلك نوصي بمزيد من الدراسة عن الخلل الوظيفي في معدلات الأيض داخل الميتوكندريا عند مرضى متلازمة الأيض مما قد يزيد من القدرة على فهمه بصورة أوضح والوصول إلى علاجات جديدة، للتمكن من إحداث التوازن بين تكوين المؤكسدات الداخلية وتكون الشقوق الحرة من جانب والتخلص منها من جانب آخر.

التلف التأكسدي للحمض النووي (دي إن إيه)
عند المرضى بمتلازمة الأيض

رسالة علمية

مقدمة لقسم الكيمياء الطبية التطبيقية

معهد البحوث الطبية ـ جامعة الإسكندرية
ايفاءا جزئيا لشروط الحصول على درجة

الدكتوراه

في

الكيمياء الطبية التطبيقية

من

عمر إبراهيم محمود الغمراوي
بكالوريوس علوم (شعبة علم حيوان)
كلية العلوم
جامعة القاهرةـ ١٩٩٦م

ماجستير الكيمياء الطبية التطبيقية
معهد البحوث الطبية
جامعة الإسكندريةـ ٢٠٠٧م

معهد البحوث الطبية
جامعة الإسكندرية

٢٠١٤م

التلف التأكسدي للحمض النووي (دي إن إيه) عند المرضى بمتلازمة الأيض

رسالة علمية مقدمة من

عمر إبراهيم محمود الغمراوي

للحصول على درجة الدكتوراه

في

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