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No gender associated differences in LDL oxidation in response to a $CuSO_4$ challenge in a population of Caucasians with well-controlled type 2 diabetes

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Abstract
It was hypothesized that there would be gender differences in low density lipoprotein (LDL) oxidation in response to a $CuSO_4$ challenge in the study population of well-controlled (HbA1c < 8 %) Caucasian type 2 diabetics. The purpose of this study was to assess this hypothesis. Males (n=18) and females (n = 14) participated in this study. Subjects came in for two visits 3 months apart. LDL was isolated by ultracentrifugation and exposed to 5 μM $CuSO_4$. There were no gender differences in lag time, maximal rate of propagation or maximal oxidation as measured by conjugated diene formation measured at 234 nm. It is concluded that gender does not play a role in $CuSO_4$-induced LDL oxidation in vitro in the study population. It may be that both genders need to much more aggressively manage dietary patterns to reduce the impact of LDL oxidation in their susceptibility to atherosclerosis-induced myocardial infarction.

Keywords: *human, LDL oxidation, dietary intakes, gender, type 2 diabetes*

Introduction

Cape Breton Island and in particular the Cape Breton regional municipality in the province of Nova Scotia, Canada suffers from among the highest rates of obesity[1] and type 2 diabetes in Canada, the consequence of which are seen in the overall economy and in the competition for healthcare dollars with other health issues. Consequently it is important to control this disease as much as possible so as to reduce its economic and social impact.

Fuller *et al*[2] have reported that polymorphonuclear cells can oxidize LDL. The susceptibility of LDL to oxidation is believed to play a role in atherosclerosis-induced myocardial infarction[3-6] though this is controversial. As LDL becomes progressively more oxidized it putatively is more readily taken into both macrophages and arterial wall cells thus increasing the rate of cholesterol influx into the arterial wall.[7-13] Some authors have suggested that in type 2 diabetics compared to healthy controls, LDL is more susceptible to oxidation and such persons have higher levels of oxidation which may contribute to the more aggressive atherosclerosis found in type 2 diabetics compared to non-diabetics.[14-28] In contrast, Makimattila *et al*[29] observed that LDL oxidation lag time (susceptibility to oxidation) did not contribute to reduced endothelial vasodilatation nor did LDL oxidation levels.[30] Reduced endothelial vasodilatation is a contributor to the atherosclerotic process. Further, Hayashi *et al*[31] have indicated that no relation exists between oxidised LDL and carotid artery thickness a measure of progression. Leinonen *et al* [32] found no relation between susceptibility of LDL to oxidation and coronary heart disease. After adjustment for lipids and lipoproteins hypertension, BMI and waist to hip ratios, differences in lag time between type 2 diabetics and healthy controls were eliminated.[33]

Conjugated diene formation results from polyunsaturated fatty acid oxidation. Conjugated diene formation on LDL, as a measure of in vivo LDL oxidation [34-35], has been linked to the increased risk of atherosclerosis is type 2 diabetics. Decreased oxidation lag time, increased conjugated diene propagation rate and increased total conjugated diene formation in copper challenged LDL have been linked to the increased risk of atherosclerosis is type 2 diabetics.[36-37]

Dietary fatty acids may impact LDL oxidation. Dietary intake of oleic acid has been associated with decreased LDL oxidation in type 2 diabetics[38] and in non-diabetics.[39] Hargrove *et al*[40] noted that higher intakes of PUFA can increase LDL conjugated diene formation.

Higher levels of oxidation have been reported in female Japanese type 2 diabetics compared to male Japanese type 2 diabetics [41] but this has never been done in Caucasian type 2 diabetics.

On the basis of higher oxidation levels being reported in female Japanese type 2 diabetics, it was hypothesised that there would be gender differences in lag time, propagation rate and maximal oxidation levels as measured by conjugated diene production. The purpose of this study was to assess this hypothesis.

Patients and Methods

Subjects

Subjects (n =18 male, 14 female) were Caucasians

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responding in approximately equal sex numbers to a Sydney, Nova Scotia newspaper advertisement and two area physicians. This study received approval from the Cape Breton University Human Ethics Review Committee. So as to determine metabolic stability subjects came for visit 1 and 3 months later for visit 2.

Inclusion criteria: 18 years of age and older; Well controlled type 2 diabetes HbA1c < 8

Exclusion criteria: Liver or kidney pathology; Hospitalisation; Taking insulin;Inability to follow the protocol

Dietary records were collected on three separate days, one at the beginning (day 1), one in the middle (day 45) and one at the end of the 90 day period between visits one and two. Dietary assessment was done by the Food Processor software.

LDL (density of 1.019-1.063) was isolated by ultracentrifugation using potassium bromide.[42] As per Fuller *et al*,[43] the isolated LDL was dialysed for 24 hours at 4 ^{o} C against 10 L of saline/EDTA(150 mmol NaCl/L, 1 mmol/L EDTA/L). LDL was then filtered and stored at 4^{o}C until protein was measured via the Lowry method using bovine serum albumin for construction of a standard curve. LDL oxidation studies were performed within 48 hours of LDL isolation. LDL oxidation was done after an overnight dialysis of the LDL against 1 L phosphate buffered saline (PBS) pH 7.4 at 4 ^{o} C. LDL (200 mg protein/L) was oxidized over an 8 hour period at 37 ^{o}C in a cell free system using 5 μmol $CuSO_4$/L in PBS pH 7.4. The time points were 0, 0.5, 1 1.5, 2, 3, 4, 5 and 8 hours.

The level of oxidation was measured as conjugated dienes at 234 nm using a Spectromax 190 plate reader (Molecular Devices, Sunnyvale, CA). The lag phase was measured by determining the intersection point between the tangent to the slope of the propagation curve and the horizontal axis. The difference between zero time and that intersection time is the lag phase. The maximal slope (i.e. rate of conjugated diene formation) is the steepest part of the slope of the propagation phase. The maximal oxidation conjugated diene formation was the difference in ΔA234nm between the maximal absorbance measured and absorbance at 0 hours.

Statistical analyses

Subject data is in table 1 and was assessed by a two way ANOVA. The data in tables 2, 3 and 4 was assessed by multivariate analysis of variance using a general linear model and comparing the visits 1 and 2 for females and again for males. The data in tables 2 and 3 was also compared for visit 1 males versus visit 1 females and for visit 1 males versus visit 2 females. The statistical analyses were done using the Minitab software (version 15).

Results

Subject data is given in table 1. Males and females were of similar age. Males were consistent in their anthropometric measures from days one to two (Table 2). Females experienced a minor drop in weight, waist and hip circumferences from visits one to two (Table 2). In table 3 it is noted that both males and females were consistent over three days of dietary intake aside from the fact that males consumed more calories than females and had a greater tendency to consume more saturated fat. As can be seen in table 4, there was no significant difference in lag time (susceptibility to oxidation), slope (maximal rate of conjugated diene propagation) or oxidation difference (conjugated diene levels at 8 hours versus time 0) when comparing visit 1 to 2 for females or for males, comparing males visit 1 to females visit 1 or comparing males visit 2 to females visit 2.

Discussion

The lag phase, propagation rate (slope) and maximal conjugated diene production are consistent with the literature [44] and in going from visits one to two (Table 4). The metabolic stability in anthropometric measures going from visit one to two (Table 2) is consistent with the consistency in diet (Table 3) and self-reported absence of change in exercise patterns. The minor though unexplained drops in waist, weight and hip measures in females going from visits one to two did not affect the consistency in oxidation measures. The lack of gender difference in oxidation differs with Japanese type 2 diabetics [41] though the reason(s) are not clear. Ikeda *et al* [41] measured oxidation by ELISA and thus there may be differences in sensitivity between the method used in this paper compared to Ikeda *et al*.[41]

The tendency toward increased saturated fatty acid consumption in males did not produce a difference in oxidation as it is increased polyunsaturated fatty acids that increase oxidation levels in type 2 diabetics.[40]

Thus it appears that the putative risk presented by LDL oxidation to the aggressive atherosclerosis experienced by well-controlled type 2 diabetics [14-28] does not differ between males and females Caucasian type 2 diabetics in the patients in this study.

It is concluded that similar diets in terms of fatty acid composition contributed to the gender similarity of lag time, slope (propagation rate) and total conjugated diene formation. Caucasian males and females who are well controlled type 2 diabetics do not differ in lag time, slope (propagation rate) and total conjugated diene formation as assessed by the protocol used in this paper. It is clear that the combination of factors (antioxidants, diet, anthropometric measures) dictating LDL oxidation susceptibility, maximal rate and maximal production) do not differ significantly by gender in the sample of type 2 diabetic Caucasians in this study.

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Table 1: Characteristics of subjects (all Caucasians) of males compared to females. Data (n = 32) is reported as mean ± standard error of the mean (S.E.M.).

	Males visit 1	Males visit 2	Females visit 1	Females visit 2	Males –mean of visits 1 and 2	Females-mean of visits 1 and 2
N	18	18	14	14	18	14
Age (years)	59.5 ± 1.7	60.7 ± 2.9	60.7 ± 2.9	60.7 ± 2.9	59.5 ± 1.7	60.7 ± 2.9

Table 2: Anthropological measures of males compared to females. Data (N = 32) is reported as mean ± standard error of the mean (S.E.M.).

	Males visit 1	Males visit 2	Males Average of Visits 1 and 2	Female Visit 1	Female Visit 2	Female Average of Visits 1 and 2
Weight (kg)	90.7 ± 3.5	90.5 ± 3.4	90.6 ± 3.4	86.6 ± 4.4	85.3 ± 4.6 fv1vs fv2 p=0.028	85.9 ± 4.5
Height (m)	1.72 ± 0.02	1.72 ± 0.02	1.72 ± 0.02	1.59 ± 0.01	1.59 ± 0.01	1.59 ± 0.01 fv12 vs mv12 p=0.000
Waist (cm) circumference	101.5 ± 2.1	102.4 ± 2.2	101.9 ± 2.2	104.0 ± 3.1	99.5 ± 3.2 fv1vs fv2 p=0.005	101.8 ± 3.1
Hip (cm) circumference	103.9 ± 1.5	102.2 ± 1.3	103.0 ± 1.4	120.5 ± 3.9 mv1vs fv1 p=0.001	115.0 ± 3.9 fv1vs fv2 p=0.001 mv2 vs fv2 p=0.007	117.8 ± 3.9 fv12 vs mv12 p=0.002
BMI (kg/m^2)	30.3 ± 0.7	30.3 ± 0.8	30.3 ± 0.7	34.3 ± 1.6 mv1vs fv1 p=0.040	33.8 ± 1.7	34.0 ± 1.6 fv12 vs mv12 p=0.050
Waist to hip ratio	0.98 ± 0.01	1.00 ± 0.01 mv1 vs mv2 p=0.005	0.99 ± 0.01	0.87 ± 0.01 mv1vs fv1 p=0.000	0.87 ± 0.01 mv2 vs fv2 p=0.000	0.87 ± 0.01 fv12 vs mv12 p=0.000
Waist to height ratio	0.588±0.009	0.593±0.010	0.591 ± 0.010	0.655 ± 0.018 mv1vs fv1 p=0.000	0.626 ± 0.020 mv2 vs fv2 p=0.000	0.640 ± 0.018 fv12 vs mv12 p=0.000

fv1 = female visit 1; fv2 = female visit 2; fv12 = average of female visits 1 and 2; mv1 = male visit 1mv2 = male visit 2; mv12 = average of male visits 1 and 2

Table 3: Dietary intakes of males compared to females.. Data (N = 32) is reported as mean ± standard error of the mean (S.E.M.).

	Males N=18 Day 1	Males N = 18 Day 2	Males N = 18 Day 3	Females N = 14 Day 1	Females N = 14 Day 2	Females N = 14 Day 3
Calories (kcal)	1565 ± 356	1898 ± 511	1786 ± 399	1342 ± 188	1230 ± 362	1333 ± 192*
Protein (g)	60.6 ± 8.0	67.5 ± 13.8	64.7 ± 9.1	70.1 ± 13.6	61.5 ± 17.8	79.7 ± 12.4
Carbohydrate (g)	164.3 ± 58.1	225.9 ± 66.5	216.1 ± 53.5	173.5 ± 38.8	173.1 ± 60.2	164.7 ± 28.4
Fat (g)	59.6 ± 17.1	81.9 ± 22.3	77.7 ± 19.9	43.1 ± 3.5	34.1 ± 11.2	41.4 ± 5.5
Saturated fat (g)	16.3 ± 4.0	24.1 ± 6.9	20.2 ± 4.5	14.1 ± 2.8	12.6 ± 4.1	13.1 ± 3.0**
Monounsaturated fat (g)	10.8 ± 3.2	20.4 ± 10.3	16.7 ± 6.9	13.7 ± 2.3	7.5 ± 2.6	10.5 ± 3.2
Polyunsaturated fat (g)	5.3 ± 1.6	8.8 ± 3.9	10.4 ± 5.5	5.5 ± 1.3	2.8 ± 0.9	5.6 ± 2.0
Fibre (g)	17.5 ± 4.6	18.8 ± 5.0	17.9 ± 4.7	15.0 ± 1.8	14.1 ± 4.5	13.8 ± 2.2

* males consumed a higher amount of calories on each of the three days compared to females (p = 0.028)

** trend toward higher consumption of saturated fat by males compared to females over the three day period (p= 0.096)

Table 4: Lag times, slopes and maximal oxidation levels of males compare to females. Data (N = 32) is reported as mean ± standard error of the mean (S.E.M.).

	Males visit 1 n = 18	Males visit 2 n = 18	Males Average of Visits 1 and 2 n = 18	Female Visit 1 n = 14	Female Visit 2 n = 14	Female Average of Visits 1 and 2 n = 14
Lag time (minutes)	129.5 ± 16.4	133.7 ± 21.5	131.5 ± 18.4	137.4 ± 25.7	131.1 ± 21.2	134.6 ± 23.7
Maximal rate (AU/min)	0.143 ± 0.018	0. 147 ± 0.038	0.145 ± 0.034	0.139 ± 0.031	0.148 ± 0.041	0. 144 ± 0.037
Maximal oxidation (AU)	0.683 ± 0.064	0.676 ± 0.055	0.681 ± 0.059	0.727 ± 0.087	0.723 ± 0.091	0.725 ± 0.089

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