Diabetic Complications Consortium

Application Title: Role of osteocalcin in mood and cognitive complications of type 2 diabetes.

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1. Project Accomplishments:

A total of 134 participants have been screened for inclusion in the study. Of these, 69 participants have completed baseline assessments, 33 completed 3 month follow-up and 29 completed 6-month follow-up. In addition to recruiting from the Diabetes and Healthy Lifestyle Program at the Toronto Rehabilitation Institute (TRI), we have expanded our recruitment to another Diabetes Program, the Sunnybrook Diabetes Education Centre (SunDEC), whereby participants receive diabetes management education and undertake exercise *ad libitum*. Measurement of baseline serum carboxylated osteocalcin (cOC) and undercarboxylated (unOC) has been performed in the first 30 participants (26 TRI, 4 SunDEC; **Table 2**). Baseline osteocalcin measurements in the second 30 participants and in follow-ups are currently underway.

From the data collected thus far, we have measured potential associations between baseline serum osteocalcin levels and patient characteristics (e.g. demographics, medications, blood glucose, cardiopulmonary fitness; **Table 1**) and investigated the relationships at baseline between serum osteocalcin (unOC and cOc), mood (**Table 3**) and cognitive function (**Table 4 & 5**). The results presented in Section 2 highlight our findings demonstrating that unOC is positively associated with verbal learning, whereby T2DM patients with lower serum unOC levels demonstrated lower verbal learning scores. These results strengthen existing preclinical and clinical data implicating the osteocalcin system in cognitive function in T2DM. Follow-up data from this study will be necessary to further explore this relationship, and investigate whether potential unOC increases due to exercise are also correlated with cognitive function.

Associations between mood and serum osteocalcin were also investigated. We found that at baseline, there was no association between serum osteocalcin (unOC and cOC) and mood as measured by the Beck's Depression Inventory II (BDI-II). This relationship will be re-assessed with the forthcoming osteocalcin measurements in the second 30 participants, as well as through investigation of potential longitudinal findings at follow-up.

The findings generated thus far have been the subject of a Master of Science thesis, submitted in 2018 within the Department of Pharmacology and Toxicology at the University of Toronto. Furthermore, findings have been disseminated through poster presentations at National conferences and an oral presentation at the Société Québécoise de lipidologie, de nutrition et de

métabolisme (SQLNM), du Réseau de Recherche en santé Cardiométabolique, Diabète et Obésité (CMDO) et du Congrès COLosSUS, 2018. Most recently, our results have supported a successful CIHR catalyst proposal, awarded to further expand this project through the investigation of "*Sex differences in bone osteocalcin response to exercise".*

Table 1: Participant characteristics and associations with unOC and cOC (Mann-Whitney U test (U) or Spearman's rho (ρ) (n=30).

Characteristic	Median or % (n)	Interquartile Range	Association with unOCN	Association with cOCN
Socio-Demographic				
Age (years)	66.00	9.00	ρ=0.179, p=0.343	ρ=0.132, 0.485
Partnered (%)	70% (21)	-	U=87.00, p=0.734	U=81.00, p=0.541
Employed (%)	30% (9)	-	U=69.00, p=0.248	U=52.00, p=0.054
Current smoking status (% smoker)	3.3% (1)	-	U=13.00, p=0.862	U=13.00, p=0.862
Education (years)	17.00	5.00	ρ = 0.057, p=0.767	ρ=0.100, p=0.601
Sex (% female)	60% (18)	-	U = 90.50, p=0.459	U=98.00, p=0.672
Ethnicity (% Caucasian)	66.7% (20)	-	U = 70.00, p=0.494	U=70.00, p=0.495
Diabetes Complications				
Retinopathy (%)	13.3% (4)	-	U = 45.50, p=0.692	U=44.00, p=0.625
Neuropathy (%)	10.0% (3)	-	U = 15.00, p=0.078	U=17.00, p=0.104
Nephropathy	6.7% (2)	-	U=19.00, p=0.454	U=1.00, p=0.025
Diabetes duration (years)	6.00	14.00	ρ = -0.169, p=0.398	ρ=-0.111, p=0.580
Laboratory Values				
HbA1c (mmol/mol)	0.069	0.012	ρ = -0.192, p=0.310	ρ=-0.342, p=0.064
Fasting glucose (mmol/L)	6.90	5.30	ρ = -0.094, p=0.634	ρ=-0.268, p=0.167
Insulin (pmol/L)	43.54	35.72	ρ = 0.197, p=0.324	ρ=-0.199, p=0.320

Characteristic	Median or % (n)	Interquartile Range	Association with unOCN	Association with cOCN
HDL cholesterol (mmol/l)	1.28	0.245	ρ = 0.193, p=0.491	ρ=0.007, p=0.980
LDL cholesterol (mmol/l)	2.20	1.18	ρ = -0.393, p=0.164	ρ=0.007, p=0.982
Triglycerides (mmol/l)	1.16	0.68	ρ = -0.615, p=0.015	ρ=-0.118, p=0.675
unOCN (ng/ml)	1.90	1.76	-	ρ=0.303, p=0.104
cOCN (ng/ml)	8.67	1.61	ρ = 0.303, p=0.104	-
Hypoglycemic drugs				
Biguanide (%)	66.7% (20)	-	U = 89.00, p=0.505	U=102.00, p=0.914
Insulin (%)	16.7% (5)	-	U = 48.50, p=0.223	U=72.00, p=1.00
Sulfonylureas (%)	16.7% (5)	-	U = 41.50, p=0.243	U=45.00, p=0.330
SLGT-2 inhibitor (%)	16.7% (5)	-	U = 47.50, p=0.404	U=42.00, p=0.254
DPP-4 inhibitor (%)	16.7% (5)	-	U = 69.00, p=0.876	U=42.00, p=0.120
Incretin mimetic (%)	6.7% (2)	-	U = 14.00, p=0.244	U=20.00, p=0.506
Physiological Characteristics (n=25)				
Anthropometric				
BMI (kg/m^2) (n=30)	34.00	10.55	ρ = -0.180, p=0.924	ρ=-0.294, p=0.115
Abdominal girth (cm)	105.50	25.25	ρ=-0.256, p=0.216	ρ=-0.521, p=0.008
Percentage body fat (%)	40.90	19.20	ρ=-0.086, p=0.682	ρ=-0.389, 0.054

Characteristic	Median or % (n)	Interquartile Range	Association with unOCN	Association with cOCN
Resting physiology				
Resting heart rate (beats per minute)	86.00	14.00	ρ=-0.254, p=0.220	ρ=-0.060, p=0.777
Resting systolic blood pressure (mmHg)	121.00	27.00	ρ=0.004, p=0.983	ρ=-0.466, p=0.019
Resting diastolic blood pressure (mmHg)	75.00	11.00	ρ=-0.037, p=0.860	ρ=-0.271, p=0.190
Cardiopulmonary fitness parameters				
VO_{2Peak} (ml/kg/min)	19.10	10.65	ρ=-0.023, p=0.914	ρ=0.297, p=0.149
Maximum systolic blood pressure (mmHg)	178.00	31.00	ρ=-0.093, p=0.658	ρ=-0.061, p=0.773
Maximum diastolic blood pressure (mmHg)	78.00	15.50	ρ=0.208, p=0.318	ρ=-0.054, p=0.799

Table 2: Osteocalcin concentrations (n=30).

	Mean ± SD	Median	IQR
unOCN (ng/ml)	1.89 ± 0.22	1.69	1.26
cOCN (ng/ml)	8.96 ± 0.60	8.54	2.37

2. Specific Aims:

Specific Aim 1: To establish a relationship between serum osteocalcin concentrations and depressive symptoms in people with T2DM. ***We hypothesize that lower serum unOC concentrations would be associated with higher Beck Depression Inventory 2nd Ed. (BDI-II) scores.***

Results: Possible confounders related to depressive symptom scores were explored. BDI-II scores were significantly associated with gender, percentage body fat and maximum systolic blood pressure (U=36.50, p=0.003) (ρ=0.429, p=0.033) (ρ=-0.479, p=0.016) respectively. There was no univariate non-parametric association between unOC (ρ=0.046, p=0.810) or cOC (ρ=0.303, p=0.104) and BDI-II scores. There was no association between BDI-II and unOC in a linear regression model controlling for age and gender (**Table 3**).

Table 3: Linear regression of the association between unOC concentrations and BDI-II scores adjusting for covariates *a-priori*, age and gender.

Characteristics (n=30)	Unstandardized Coefficients B	Standard Error (SE)	Standard Coefficients Beta (β)	t	Sig. (p)
Unadjusted model					
unOC	1.089	1.877	0.109	0.580	0.566
Adjusted for *a-priori* covariates					
unOC	0.839	1.556	0.084	0.539	0.594
Age (years)	-0.466	0.210	-0.348	-2.218	0.036
Gender (% female)	-13.663	3.769	-0.569	-3.625	<0.001

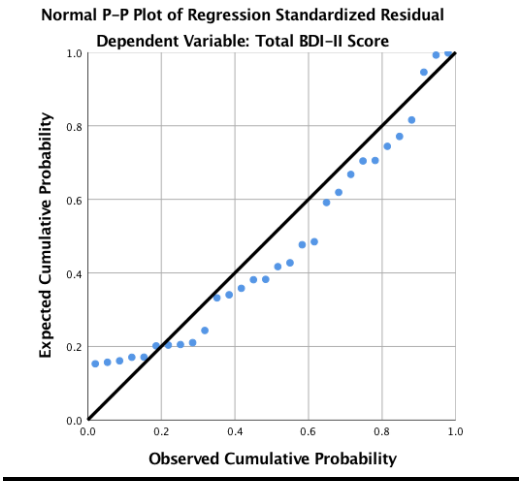


Figure 1: Probability plot of the linear regression of BDI-II scores and unOC concentrations

<u>Specific Aim 2:</u> To establish a relationship between the antidepressant benefits of physical activity and changes in unOC in people with T2DM. ***We hypothesize that a) exercise will increase serum levels of unOC, b) exercise will decrease BDI-II scores, and c) the increase in unOC will correlate with the decrease in BDI-II scores.***

Results: We have completed 6-month follow-up assessments in 29/60 participants. Serum Osteocalcin measurements in these participants are currently underway and follow-up assessments are ongoing. These data will allow us to assess potential increases in serum Osteocalcin levels and decreases in BDI-II scores relevant to exercise, and potential correlation between these factors.

<u>Specific Aim 3:</u> To explore relationships between osteocalcin and cognitive function using a standardized battery of cognitive assessments. ***We hypothesized that the increase in unOC due to exercise will correlate with the increase in cognitive scores. An important corollary is that those who do not increase their unOC will respond less, identifying a potential mediator of "exercise futility" in T2DM.***

Results: Collection of 6-month follow-up data to assess exercise-related changes in osteocalcin and cognition are currently underway, with 29/60 participants having completed 6-month follow-up assessments. Baseline relationships between osteocalcin and cognitive tests were measured by linear regression and are presented in **Tables 4 and 5** below. We administered the California Verbal Learning Test 2nd Ed (CVLT-II) to all 30 participants and compared their results to their measured serum unOC. In models controlling for age and gender, we found that in people with T2DM, unOC (β=0.415, p=0.022) but not cOC (β=0.018, p=0.928) was associated with verbal learning, but not verbal memory (**Table 5**).

Table 4: Correlation between cognitive tests and unOCN controlling for age and gender

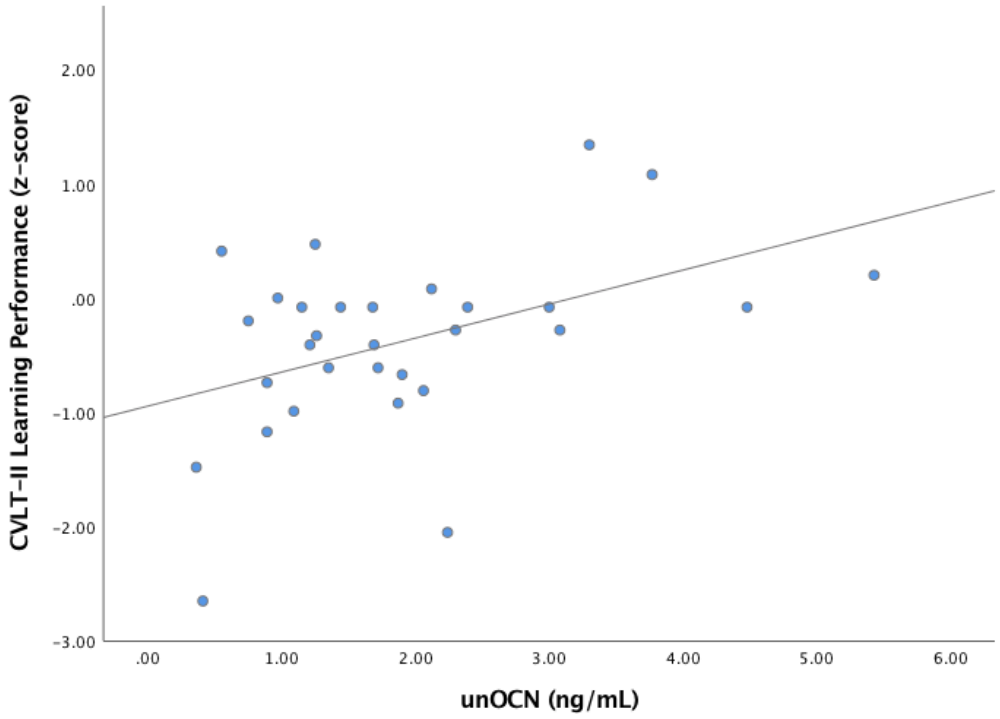
Cognitive Test	**Mean raw score ± SD**	**Mean z-score ± SD**	**p-value***
Trail Making Test A (sec)	41.11 ± 22.22	-0.18 ±1.17	0.894
Trail Making Test B (sec)	104.14 ± 35.76	-0.47 ±0.86	0.657
Digit Symbol Coding Test (symbols)	57.04 ± 11.14	-0.05 ±0.80	0.963
Victoria Version of the Stroop Neurological Screening Test, Dots (seconds)	16.43 ± 6.53	-0.95 ± 1.07	0.434
Victoria Version of the Stroop Neurological Screening Test, Words (seconds)	20.95 ± 9.27	-0.85 ± 0.97	0.880
Victoria Version of the Stroop Neurological Screening Test, Colours (seconds)	36.56 ±12.81	-0.39 ± 1.03	0.722
Controlled Oral Word Association Task (words)	35.20 ± 9.67	-0.37 ± 1.15	0.731
California Verbal Learning Test 2nd Edition-Total learning (words)	40.27±8.29	-0.38±0.80	0.017
California Verbal Learning Test 2nd Edition-Short Delay Free Recall (words)	7.59±2.85	-0.47±0.95	0.492
California Verbal Learning Test 2nd Edition-Long Delay Free Recall (words)	8.20±3.58	-0.53±1.14	0.223

** raw scores adjusted for age and gender*

Table 5: Association between CVLT-II performance and unOCN concentrations. Models controlled for age and gender.

Test	β	p
CVLT-II Learning (Trials 1-5) z-score	0.415	0.022
CVLT-II Recall (Short) z-score	0.088	0.653
CVLT-II Recall (Long) z-score	0.232	0.215

Figure 2 Correlation between serum unOCN concentrations and CVLT-II verbal learning z-scores.



3. Publications:

Thesis:
Carter Jasmine. Serum undercarboxylated Osteocalcin is associated with verbal learning in adults with Type 2 diabetes. Master of Science Thesis, Department of Pharmacology and Toxicology, University of Toronto. 2018.

Poster presentations:
Darwish L, Mitchell J, Carter J, Sugamori KS, Oh PI, Swardfager W. Circulating osteocalcin and verbal memory performance in people with type 2 diabetes mellitus. Visions in Pharmacology Research Day, 2018.

Darwish L, Mitchell J, Carter J, Sugamori KS, Oh PI, Swardfager W. Circulating osteocalcin and verbal memory performance in people with type 2 diabetes mellitus. U of T Collaborative Program in Neuroscience Research Day 2018.

Darwish L, Mitchell J, Carter J, Sugamori KS, Oh PI, Swardfager W. Circulating osteocalcin and verbal memory performance in people with type 2 diabetes mellitus. International Congress of Neuroendocrinology, 2018. Accepted.

Oral presentations:

Swardfager W. Behavioural activation in people with type 2 diabetes undertaking an exercise-based rehabilitation intervention. Société Québécoise de lipidologie, de nutrition et de métabolisme (SQLNM), du Réseau de Recherche en santé Cardiométabolique, Diabète et Obésité (CMDO) et du Congrès COLosSUS, Quebec, 2018. ***Invited oral presentation, presenting author.***

Manuscripts in preparation:

Darwish L, Eakin K, Saleem M, Sugamori K, Herrmann N, Mitchell JA, Lanctôt KL, Swardfager W. Serum osteocalcin in coronary artery disease; relationships with type 2 diabetes, body mass index, fitness and metabolic control.