Research Article

Plasma fatty acids as markers for desaturase and elongase activities in spinal cord injured males

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Objective: To investigate the use of surrogate plasma fatty acid analysis to provide further insights into the underlying adiposity and the development of metabolic syndrome in men with spinal cord injury (SCI).

Design: Case-control, cross-sectional study.

Setting: Community-based individuals with spinal cord injury and healthy controls.

Participants: Twenty men with SCI age, height and weight matched with 20 able-bodied controls.

Outcome Measures: Lean tissue (LTM) and fat mass (FM) were determined using dual energy X-ray absorptiometry. Fasting blood samples were taken for analysis of fatty acids, adiponectin, insulin, glucose and leptin. Enzymatic indices were calculated using relevant fatty acids.

Results: Total FM, leptin, stearoyl coenzyme A desaturase (SCD) $\Delta 9$ (SCD-16, 16:1/16:0, and SCD-18, 18:1/18:0) indices and $\Delta 6$ desaturase index were significantly higher ($P < 0.05$) in the SCI group than the controls. Significant differences between the groups was observed for several individual fatty acids. Correlational analysis revealed a different pattern between blood biomarkers and indices of SCDs, de novo lipogenesis and elongase. Associations between the desaturase and elongase indices and biomarkers in the controls followed those reported elsewhere for able bodied participants; the same associations were not observed in the SCI group.

Conclusion: We have identified disturbances in fatty acid biosynthesis in SCI individuals likely associated with the development of adipose tissue below the lesion and a decrease in LTM. Loss of LTM may disturb the normal skeletal muscle-fatty acid metabolic processes leading to the disruption of metabolic homeostasis, previously identified in persons with SCI.

Key words: Fatty acids, Desaturase, Elongase, Body composition, Metabolic markers

Introduction

Spinal cord injury (SCI), paralysis and reduced physical activity combine to produce significant changes in body composition^{1,2} frequently leading to an increased incidence of coronary heart disease, type II diabetes mellitus and metabolic syndrome.^{3,4} Previously we have demonstrated that the use of body mass index (BMI) on its own is a poor indicator of body fat, underestimating adiposity in SCI group² despite these subjects not appearing to be obese. Subsequent work using factor analysis of the metabolic syndrome in SCI males demonstrated interactions between adiposity (as determined by dual energy X-ray absorptiometry - DXA), plasma insulin,

glucose and dyslipidemia.⁵ More recently a loss of cross-talk between fat mass, osteocalcin, glucose metabolism and adiponectin was identified and the decentralization of the sympathetic nervous system (SNS) was proposed as a potential causative mechanism for this loss.^{6,7} As a key regulator of energy metabolism the SNS is involved in the control of fat mass, insulin sensitivity via osteocalcin, leptin and adiponectin.⁶ While dietary fat has an important role in obesity development, metabolic syndrome and associated disorders, and endogenous fat synthesis by adipose tissues is now known to be important as a causative metabolic disease factor.^{8,9} It has been previously demonstrated that high plasma levels of palmitic acid (C16:0), low levels of linoleic acid (C18:2) and higher levels of

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palmitoleic acid (C16:1) are metabolic characteristics for insulin resistance and metabolic syndrome;¹⁰ however, to date no information exists on the relationship of individual fatty acids and persons with SCI.

Metabolic control of both exogenous and endogenous fatty acids are regulated, in part, by stearyl-CoA desaturase isoenzymes and elongases, that are responsible for converting palmitic acid (C16:0) into longer chain fatty acids for further use in the body.¹¹ A key enzyme in this process is stearyl-CoA desaturase-1, which catalyzes the conversion of C16 and C18 fatty acids into C16:1 (palmitoleic acid) and C18:1 (oleic acid), respectively.^{11,12} Although it is not possible to measure this enzyme in humans, surrogate analytes have been identified which reflect the activity of the stearyl-CoA desaturase activity and a 'desaturation index' may be calculated by using ratios of plasma fatty acids e.g. C16:1/C16:0 is a surrogate measure of $\Delta 9$ desaturase activity; C18:3/C18:2, $\Delta 6$ desaturase activity; C18:0/C16:0 elongase activity; C16:0/C18:2 de-novo lipogenesis; C20:n6/C20:4n6 stearyl-CoA desaturase $\Delta 5$ and stearyl-CoA desaturase $\Delta 6$ activity.^{11,13} The utility of the plasma fatty acid ratios in predicting metabolic de-arrangements and the linkages associated with metabolic syndrome, atherosclerosis and diabetes mellitus are now well established in able bodied persons^{9,14,15} and provide additional insights in the metabolic mechanisms underlying these disease processes. We have utilised a novel approach by using surrogate markers of plasma fatty acid metabolism to provide further insights into the underlying adiposity and disease processes in SCI individuals.

Participants and methods

Subjects

Twenty men (aged between 16 and 52 years) who had sustained a traumatic injury to the spinal cord participated in this study, were height and weight matched with non-injured controls. All participants had to be 16 years or over, could provide informed consent, did not have diagnosed diabetes, thyroid problems or heart disease, had stable body weight (no gains or losses of > 5 kg in the previous 6 months), were not restricting any specific food group and considered to be consuming a 'balanced' diet. In addition, the SCI group had to have sustained their injury for more than one year, which is indicative of chronic spinal cord injury. Participants with SCI were recruited through the Burwood Spinal Injury Unit, Canterbury and the able bodied controls were recruited via advertisement. Using the American Spinal Injury Association

classification system,¹⁶ thirteen participants were tetraplegic. Five men with lesions between C4-C7 were AIS A, six AIS B, one AIS C and one AIS D. Seven paraplegic participants (T5-L3) had sustained a spinal cord lesion between T5-L3; four were AIS A, one AIS B; and two AIS C. The Health Funding Authority, Otago and Canterbury Ethics Committees approved the study.

Data collection

Total body mass, total, leg and trunk fat mass, total, leg and trunk lean tissue mass (LTM) (FM) and body fat percentage were determined using dual energy X-ray absorptiometry (DXA) as previously described.² Due to the difficulty in obtaining height in persons with SCI, we utilised the ruler function in the DXA software for height measurement in all participants. All participants were asked to abstain from physical activity for 24 hours and food or fluid (other than water) for 12 hours prior to testing. Participants arrived at the testing laboratory between 8:00am and 10:00 am and an oral glucose tolerance test was undertaken to assess carbohydrate metabolism. Briefly, 75g of a glucose polymer powder (Polycose, Ross Products Division, Abbott Laboratories, Ohio, USA) was dissolved in 300ml of water at room temperature. Following the insertion of a cannula into a vein in the antecubital fossa, blood was drawn for baseline analysis. Each participant was then asked to consume the glucose solution within a five minute period. Blood was then drawn at 30, 60, 90 and 120 minutes for analysis of insulin and glucose. Area under the curve (AUC) was calculated for both glucose and insulin. Fasting blood samples were analysed for fatty acids, adiponectin, insulin, glucose and leptin. Plasma was separated and frozen at -80°C until analysis.

Physical activity was collected using an in-house questionnaire based on existing physical activity questionnaires. The questionnaire asked participants to state what activities they performed and the duration of each session of the activity undertaken. The sum of the activities provided the total minutes of physical activity per week. An in-house questionnaire was used, as it provided the opportunity to include wheelchair activities. The questionnaires were adapted from the Godin-Shepherd Physical Activity Questionnaire¹⁷ and are available as supplemental material.

Adiponectin and leptin were analyzed by radioimmunoassay using commercial kits (Linco Research, St Charles, MO, USA) by Endolab, Christchurch, New Zealand. Insulin was analyzed by radioimmunoassay (Diagnostic Products Corporation, Indianapolis, USA)

and glucose using the hexokinase method on a Roche Cobas Mira Plus analyzer (Roche Diagnostics, Corporation, Indianapolis, USA) in the Human Nutrition Department, University of Otago, New Zealand. HOMA-IR and the Matsuda insulin sensitivity index (Matsuda ISI) were calculated as previously described.^{18,19}

Fatty acid analysis

Fatty acids were extracted from one ml of fasting plasma according to the method of Bligh and Dyer (1959).²⁰ Briefly, 3.75 ml of chloroform and methanol (2:1) was added to one ml of plasma and mixed by a vortex mixer for one minute. The samples were then centrifuged at 1500g for five minutes and the supernatant retained. The resulting pellet was re-suspended in one ml of water then re-extracted as described above. Following centrifugation the two supernatants were combined and 2.5ml of chloroform with 0.08% sodium chloride (by weight) in water were added within two minutes of vortexing after each addition, then centrifuged for 10 minutes at 1500g to separate the phases. The lower chloroform layer containing the fatty acids was removed and stored at -20°C overnight.

For fatty acid methylation, the chloroform layer was evaporated to dryness under a stream of oxygen free nitrogen and reconstituted in 50µl chloroform. A water (blank) sample was extracted under identical conditions. The extracts were then methylated using 3ml of a 6% (by volume) sulphuric acid in methanol. Acid-catalyzed trans-esterification was at 80°C for 12 hours after which the samples were cooled to room temperature and 2ml of hexane followed by 1ml of water was added and mixed by vortex mixer. The upper hexane layer containing the fatty acid methyl-esters was removed and stored at -20°C until analysis.

The analysis of the fatty acid methyl esters was based on the previously described method described by Holub and Skeaff (1987)²¹ and Hodson *et al.* (2004).²² Briefly, prior to analysis by gas chromatography the fatty acid containing hexane was evaporated to dryness under a stream of oxygen free nitrogen and reconstituted in 45µl of hexane. The fatty acid methyl esters were separated using a DB225 capillary column (30mx0.53mm; id 0.25µm film) obtainable from Agilent Technologies, USA. The gas chromatograph (HP 5890) was equipped with and autosampler (HP7673) and a Chem Station integration (Hewlett Packard, Avondale, PA). Following injection the column was held at an isothermal temperature of 190°C for 30 minutes and fatty acid peaks were identified by retention time compared with matching authenticated fatty acid standards

(NuCheck Prep, Elyson, Minnesota, USA and Sigma Chemical Co, St Louis USA). All results were transferred using a Microsoft Excel macro programme into a spreadsheet for further analysis.

Statistical analysis

Means and standard error of the mean (SEM) were calculated for all measured variables. Shapiro-Wilk tests were performed to ascertain normality of distribution for all data. The measures that violated normality of distribution included, glucose, insulin, leptin and all fatty acids other than myristic (14:0), pentadecanoic (15:0), oleic (18:0), linoleic (18:2n-6), and docosatetraenoic (22:4n-6) acids. All variables identified were log transformed (base 10) for analysis; for ease of understanding, non-log transformed means and SEM are presented. Independent t-tests were used to analyse differences between the SCI and control groups on all variables, while Pearson product moment correlations were used to identify associations between fatty acid desaturases and metabolic markers and body composition variables. Partial correlations were performed for all significant associations identified in both groups, controlling for age. All analyses were undertaken using the Statistical Package for Social Sciences (SPSS) v22.0 (IBM Corp., Armonk, NY). Statistical significance was accepted at $P < 0.05$. Cohen's *d* effect sizes (ES) were calculated for all statistically significant t-test comparisons, using $M_2 - M_1 / SD_{\text{pooled}}$. Where M_2 = able-bodied control group and M_1 = SCI group and $SD_{\text{pooled}} = \sqrt{((SD_1^2 + SD_2^2) / 2)}$. Small, medium, and large ES (Cohen's *d*) were identified as 0.2, 0.5, and 0.8, respectively.²³

Table 1. Group characteristics for spinal cord injured males and able-bodied controls (mean ± SEM).

Parameter	SCI (n=20)	Controls (n=20)	Significance (P value)
Age (years)	33 ± 2	33 ± 2	0.9
DXA Height (cm)	180 ± 1	179 ± 1	0.6
Weight (kg)	75.8 ± 3.1	77.2 ± 1.6	0.7
DOI (years)	10.3 ± 1.8	NA	NA
BMI	23.5 ± 1.0	24.1 ± 0.4	0.6
Total FM (kg)	21.6 ± 2.4	13.9 ± 1.3	0.008
Body fat (%)	27.5 ± 2.3	17.8 ± 1.5	0.001
Leg fat (kg)	7.0 ± 0.8	4.9 ± 0.4	0.018
Trunk fat (kg)	11.2 ± 1.3	7.1 ± 0.8	0.011
Total LTM (kg)	51.4 ± 1.9	60.0 ± 1.1	0.0005
Leg LTM (kg)	14.6 ± 0.8	21.6 ± 0.5	0.0005
Trunk LTM (kg)	25.9 ± 0.9	27.0 ± 0.5	0.250
Physical activity (mins/wk)	375.5 ± 58.6	311.5 ± 45.5	0.394

SCI, spinal cord injury group; DXA, dual energy X-ray absorptiometry; NA, not applicable; DOI, duration of injury; BMI, body mass index; FM, fat mass; LTM, lean tissue mass.

Results

Demographic and body composition details for all participants are presented in Table 1. There were no significant differences ($P > 0.05$) between the groups for age, height, weight, BMI or total minutes of physical activity per week. Thirteen of the 20 SCI participants were wheelchair athletes at either the regional (provincial) or national (New Zealand) level and as such were highly physically active. Total FM ($d = 0.89$), percent body fat ($d = 1.12$), trunk FM ($d = 0.85$) and leg FM ($d = 0.76$) were significantly higher ($P < 0.05$) in the SCI group than the controls, while total and leg LTM ($d = 1.27$ and 2.47 , respectively) was lower. There was no difference between the groups for trunk LTM values ($d = 0.35$). Plasma levels of leptin ($d = 0.92$), insulin and glucose AUC ($d = 1.08$ and 1.31 , respectively) were significantly higher ($P < 0.05$) in the SCI group (Table 2). The Matsuda ISI was significantly lower in the SCI group and a large effect size was noted ($d = 1.12$). However adiponectin, fasting insulin, fasting glucose, and HOMA IR were not significantly different ($P > 0.05$) between the SCI and control groups. However, post-load (two hour) plasma glucose and insulin were significantly higher in the SCI group (Table 2), with large effect sizes, $d = 1.26$ and 1.15 , respectively.

Significant differences ($P < 0.05$) between the groups was observed for several individual fatty acids (Table 3), with small to medium effect sizes for the majority of those fatty acids reaching significance. A large effect was observed for palmitic and eicosadienoic acids. Of the saturated fatty acids, myristic (14:0, $d = 0.67$) and palmitic (16:0, $d = 0.82$) acids were higher in the controls compared with the SCI group (Table 3). SCI participants had higher levels of the monounsaturated fatty acids, 5-dodecenoic (12:1, ES = 0.50), palmitoleic

Table 3. Weight percent content (mean $\pm$ SEM) of fatty acids (g/100g) in SCI and control groups.

Fatty acid	SCI	Control	Significance (P value)
SFA			
12:0	0.057 $\pm$ 0.010	0.101 $\pm$ 0.021	0.061
14:0	0.955 $\pm$ 0.081	1.185 $\pm$ 0.074	0.041
15:0	0.245 $\pm$ 0.019	0.275 $\pm$ 0.019	0.256
16:0	21.795 $\pm$ 0.508	23.422 $\pm$ 0.374	0.030
18:0	7.070 $\pm$ 0.244	7.432 $\pm$ 0.194	0.850
20:0	0.261 $\pm$ 0.017	0.258 $\pm$ 0.010	0.449
22:0	0.693 $\pm$ 0.048	0.686 $\pm$ 0.034	0.646
24:0	0.606 $\pm$ 0.046	0.583 $\pm$ 0.040	0.499
MUFA			
12:1	0.055 $\pm$ 0.008	0.039 $\pm$ 0.006	0.040
14:1	0.074 $\pm$ 0.034	0.091 $\pm$ 0.022	0.152
16:1	3.124 $\pm$ 0.209	2.783 $\pm$ 0.119	0.027
18:1	23.560 $\pm$ 0.567	22.288 $\pm$ 0.562	0.119
20:1	0.217 $\pm$ 0.014	0.238 $\pm$ 0.010	0.387
22:1	0.133 $\pm$ 0.018	0.120 $\pm$ 0.027	0.194
24:1	1.185 $\pm$ 0.085	0.977 $\pm$ 0.058	0.006
n-6 PUFA			
18:2 n-6	22.635 $\pm$ 0.649	25.024 $\pm$ 0.698	0.017
18:3 n-6	1.051 $\pm$ 0.679	0.426 $\pm$ 0.033	0.245
20:2 n-6	0.169 $\pm$ 0.012	0.219 $\pm$ 0.015	0.027
20:3 n-6	1.207 $\pm$ 0.082	1.047 $\pm$ 0.081	0.047
20:4 n-6	0.091 $\pm$ 0.009	0.074 $\pm$ 0.010	0.107
22:2 n-6	0.028 $\pm$ 0.004	0.046 $\pm$ 0.008	0.049
22:4 n-6	0.158 $\pm$ 0.017	0.146 $\pm$ 0.014	0.589
22:5 n-6	0.065 $\pm$ 0.015	0.066 $\pm$ 0.012	0.812
n-3 PUFA			
18:3 n-3	0.571 $\pm$ 0.123	0.529 $\pm$ 0.048	0.267
18:4 n-3	0.180 $\pm$ 0.018	0.141 $\pm$ 0.011	0.021
20:3 n-3	0.142 $\pm$ 0.042	0.173 $\pm$ 0.010	0.017
20:4 n-3	0.091 $\pm$ 0.009	0.074 $\pm$ 0.010	0.441
20:5 n-3	0.620 $\pm$ 0.096	0.642 $\pm$ 0.064	0.194
22:5 n-3	0.102 $\pm$ 0.052	0.033 $\pm$ 0.023	0.317
22:6 n-3	1.737 $\pm$ 0.148	1.527 $\pm$ 0.010	0.117

SCI, spinal cord injured; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SEM, standard error of the mean.

(16:1, $d = 0.45$) and nervonic (24:1, $d = 0.64$) acid. In the polyunsaturated n-6 group, dihomogamma-linoleic acid (20:3n-6, $d = 0.44$) was higher in the SCI group,

Table 2. Blood measures comparison for spinal cord injured and control groups (mean $\pm$ SEM).

Parameter	SCI (n=20)	Controls (n=20)	Significance (P value)
Leptin (μ g/l)	6.1 $\pm$ 1.1	2.7 $\pm$ 0.4	0.002
Adiponectin (mg/l)	11.9 $\pm$ 1.2	9.6 $\pm$ 1.0	0.151
Fasting insulin (μ U/ml)	8.1 $\pm$ 1.0	7.2 $\pm$ 0.7	0.557
2hr insulin (μ U/ml)	64.0 $\pm$ 11.9	19.3 $\pm$ 3.3	0.0005
Insulin AUC (μ U/ml)	7801.1 $\pm$ 870.7	3903.5 $\pm$ 354.5	0.002
Fasting glucose (mmol/l)	5.2 $\pm$ 0.1	5.5 $\pm$ 0.1	0.077
2hr glucose (mmol/l)	7.2 $\pm$ 0.6	4.5 $\pm$ 0.3	0.0005
Glucose AUC (mmol/l)	893.9 $\pm$ 51.8	689.0 $\pm$ 30.7	0.0005
HOMA IR	1.8 $\pm$ 0.2	1.8 $\pm$ 0.2	0.734
Matsuda ISI	4.8 $\pm$ 0.4	7.6 $\pm$ 0.7	0.001

SCI, spinal cord injury group; AUC, area under the curve; HOMA IR, homeostatic model assessment Insulin resistance; ISI, insulin sensitivity index. Conversion factors: leptin, μ g/l $\times$ 0.0625 = nmol/l; insulin, μ U/ml $\times$ 6.945 = pmol/l; conversion factor not available for adiponectin.

while linoleic (18:2n-6, $d = 0.79$), eicosadienoic (20:2n-6, $d = 0.82$) and docosadienoic (22:2n-6, $d = 0.62$) acids were higher in the control group. Two fatty acids reached significance between the groups, with stearidonic acid (18:4n-3, $d = 0.58$) higher in the SCI group and eicosatrienoic acid (20:3n-3, $d = 0.22$) higher in the controls.

Both stearoyl coenzyme A desaturase (SCD) $\Delta 9$ indices (16:1/16:0, SCD-16, and 18:1/18:0, SCD-18) were significantly higher ($d = 0.66$ and 0.79 , respectively) in the SCI group compared with the able bodied controls (Table 4). Similarly, the $\Delta 6$ desaturase index (20:3n-6/18:2n-6, $d = 0.24$) was also significantly higher in the SCI group (Table 4).

In the SCI group, a negative association between the enzyme activity of SCD-18 and adiponectin and a positive association between SCD-18 and glucose AUC was observed (Table 5). These associations with SCD-18 remained significant after controlling for age ($r = -.629$, $P = 0.004$ and $r = .460$, $P = 0.048$, adiponectin and glucose AUC, respectively). A significant negative association between elongase and glucose AUC was

noted, while the association between elongase and adiponectin approached significance (Table 5). The association between elongase and glucose AUC remained significant after age was included in the analysis ($r = -.502$, $p = 0.028$). De novo lipogenesis (16:0/18:2) was positively and significantly associated with post-load insulin and glucose, and negatively associated with physical activity. Post-load glucose was no longer significantly associated with de novo lipogenesis after controlling for age, $r = .448$, $P = 0.054$; however, associations with both post-load insulin and physical activity remained significant ($r = .563$, $P = 0.012$ and $r = -.549$, $P = 0.015$, respectively). A negative association between de novo lipogenesis and LTM and positive association with leptin, with these approaching significance at $P < 0.06$. Negative and significant associations between the SCD $\Delta 5$ index and fasting insulin and HOMA-IR was found (Table 5). The correlation for SCD $\Delta 5$ index with fasting insulin ($r = -.555$, $P = 0.014$) and HOMA-IR ($r = -.507$, $P = 0.027$) strengthened and remained significant after controlling for age.

Table 4. Mean and SEM of fatty acid ratios used to reflect measures of enzymatic activity.

Parameter	SCI (n=20)	Controls (n=20)	Significance (P value)
Stearoyl Coenzyme A desaturase $\Delta 9$ (16:1/16:0)	0.140 $\pm$ 0.009	0.119 $\pm$ 0.005	0.043
Stearoyl Coenzyme A desaturase $\Delta 9$ (18:1/18:0)	3.407 $\pm$ 0.129	3.022 $\pm$ 0.081	0.016
Elongase (18:0/16:0)	0.323 $\pm$ 0.008	0.318 $\pm$ 0.008	0.662
16:1/18:0	0.433 $\pm$ 0.031	0.382 $\pm$ 0.022	0.189
De novo lipogenesis (16:0/18:2)	0.976 $\pm$ 0.034	0.958 $\pm$ 0.043	0.744
Stearoyl Coenzyme A desaturase $\Delta 5$ (20:4n-6/20:3n-6)	4.330 $\pm$ 0.341	4.520 $\pm$ 0.343	0.697
Stearoyl Coenzyme A desaturase $\Delta 6$ (20:3n-6/18:2n-6)	0.053 $\pm$ 0.004	0.042 $\pm$ 0.014	0.029

Table 5. Pearson product moment correlations between markers of enzyme activity and body composition, adipocytokines, insulin and insulin resistance for SCI group (n=20).

	16:1/16:0	18:1/18:0	18:0/16:0	16:1/18:0	16:0/18:2	20:4n6/ 20:3n6	20:3n6/ 18:2n6
Body fat (kg)	.070	.310	-.338	.141	.305	-.261	-.034
BMI	.034	.043	-.047	.045	.073	-.228	.034
Adiponectin	.360	-.508*	.435 [#]	.194	.276	.332	.000
LTM	-.159	-.154	.164	-.187	-.417 [#]	-.121	-.074
Leptin	.169	.314	-.377	.247	.430 [#]	-.091	.009
FPI	.005	-.005	.069	-.024	.152	-.494*	.310
2hr insulin	.363	.073	-.146	.397	.577**	.133	.390
AUC insulin	.365	.181	-.141	.415	.383	.072	.250
HOMA IR	.012	-.014	.083	-.024	.177	-.466*	.260
Matsuda ISI	-.121	-.351	.273	-.270	-.353	.268	-.249
2hr glucose	.154	.270	-.392	.215	.530*	-.069	.097
AUC glucose	-.013	.466*	-.513*	.090	.301	-.297	.103
Phys Act	-.350	-.096	.050	-.346	-.602**	-.116	-.259

BMI, body mass index; LTM, lean tissue mass; FPI, fasting plasma insulin; HOMA IR, homeostasis model assessment-insulin resistance; AUC, area under the curve; ISI, insulin sensitivity index; Phys Act, physical activity. Insulin, glucose, and leptin log transformed. * $P < 0.05$; ** $P < 0.01$; [#]trend at $P < 0.06$.

Table 6. Pearson product moment correlations between markers of enzyme activity and body composition, adipocytokines, insulin and insulin resistance for controls (n=20).

	16:1/16:0	18:1/18:0	18:0/16:0	16:1/18:0	16:0/18:2	20:4n6/ 20:3n6	20:3n6/ 18:2n6
Body fat (kg)	.127	.242	-.189	.212	.708**	-.201	.122
BMI	.104	.215	-.399	.232	.541*	-.239	.245
LTM	-.163	-.153	-.082	-.069	-.223	-.268	-.120
Adiponectin	-.367	-.336	.286	-.384	-.243	-.015	-.309
Leptin	.179	.353	-.203	.249	.511*	-.172	.140
FPI	.447*	.585**	-.632**	.604**	.223	.165	.452*
2hr insulin	.133	.271	-.121	.163	.127	.172	.132
AUC insulin	-.316	.027	.099	-.242	.136	-.120	.070
HOMA IR	.339	.503*	-.565**	.501*	.296	.149	.434#
Matsuda ISI	.132	-.165	.144	-.003	-.209	.128	-.199
2hr glucose	.396	.181	-.110	.345	.411	-.064	.190
AUC glucose	-.191	-.228	.487*	-.345	.139	-.034	.025
Phys Act	.149	-.116	-.257	.202	-.666**	.012	.007

BMI, body mass index; LTM, lean tissue mass; FPI, fasting plasma insulin; HOMA IR, homeostasis model assessment-insulin resistance; AUC, area under the curve; ISI, insulin sensitivity index; Phys Act, physical activity. Insulin, glucose, and leptin log transformed. *P < 0.05; **P < 0.01, #trend at P = 0.06.

Several associations were observed in the control group (Table 6). De novo lipogenesis was positively correlated with body fat, BMI and leptin, and negatively associated with physical activity. Significance between de novo lipogenesis and BMI was lost after controlling for age ($r = .397$, $P = 0.092$) and also with leptin ($r = .370$, $P = 0.119$), but remained significant for body fat ($r = .579$, $P = 0.009$) and physical activity ($r = -.612$, $P = 0.005$). Fasting insulin was positively correlated with the SCD-16 and SCD-18 and $\Delta 6$ desaturation indices, and 16:1/18:0. Fasting insulin remained significantly associated with SCD-16 ($r = .489$, $P = 0.033$), SCD-18 ($r = .586$, $P = 0.008$), and 16:1/18:0 ($r = .631$, $P = 0.004$) after controlling for age, but the association with the $\Delta 6$ desaturation index was lost ($r = .419$, $P = 0.074$). The association between HOMA-IR and SCD-18 index (18:1/18:0) and 16:1/18:0 was positive and significant ($P < 0.05$), with the $\Delta 6$ -desaturase and HOMA-IR association approaching significance ($P < 0.06$). Associations between HOMA-IR and SCD-18 ($r = .509$, $P = 0.026$) and 16:1/18:0 ($r = .544$, $P = 0.016$) remained significant. Elongase was positively associated with glucose AUC and negatively associated with fasting insulin and HOMA-IR (Table 6). All associations remained significant following age adjustment, $r = .548$, $P = 0.015$, $r = -.632$, $P = 0.004$ and $r = -.571$, $P = 0.011$, respectively).

Discussion

The results from this study indicate that there are significant differences between serum fatty acids for the SCI and control groups in the four major groups of fatty acids investigated (saturated fatty acids, SFA; monounsaturated fatty acids, MUFA; n-6 polyunsaturated

fatty acids, n-6 PUFA, and n-3 PUFA). Taking these results into consideration, we investigated the inter-relationship of the fatty acid desaturases and elongase activities between both groups. High concentrations of palmitic acid (C16:0) and low concentrations of linoleic acid (C18:2 n-6) and proportionately elevated palmitoleic acid (C16:1) have been described as characteristic of individuals with high insulin levels and at risk for metabolic syndrome.^{11,14} Previously, it has been demonstrated that stearoyl – CoA desaturase (a liver microsomal enzyme) is the rate limiting step in the biosynthesis of palmitoleoyl and oleoyl CoAs from their respective substrates palmitoyl and stearoyl CoAs, via a $\Delta 9$ desaturation reaction.^{24,25} However, direct analysis of this enzyme in human material is difficult and the ratios of the fatty acids oleate (C18:1/stearate (C18:0) and palmitoleate (C16:1)/palmitate (C16:0) are reliable analytes to indicate surrogate enzyme activity.^{9,12}

A number of findings result from this work, not previously described for the SCI population. The fatty acid markers for stearoyl coenzyme A desaturases $\Delta 9$ and $\Delta 6$ activities are consistent with previous research, which was identified in obese individuals with metabolic syndrome.^{25,26} Stearoyl coenzyme A desaturase $\Delta 5$ has been reported as decreased in metabolic syndrome,^{9–11} which we did not find in this work. Additionally, we identified that stearoyl coenzyme A desaturase $\Delta 6$ was significantly higher than the controls. The relationship of $\Delta 5$ and $\Delta 6$ desaturase activities has been linked to the development of type 2 diabetes,¹³ an association that is consistent with our data. Compared to the controls, the SCI group had significantly higher insulin and glucose bioavailability (AUC), two hour insulin

and blood glucose, predictive markers of the development of type 2 diabetes in other populations.^{27,28} These results are considered to be indicative of type 2 diabetes and metabolic syndrome risk and are consistent with the significant decrease in lean tissue mass (LTM), despite the BMI data not being significantly different. Previously we have identified that despite the similarity of the BMI between SCI men and controls, there was up to 47% increase of total fat mass in the SCI group, with a corresponding 16% decrease in LTM and a 12% decrease in bone density.¹ Subsequently, using factor analysis we identified that while BMI remained within normal limits the SCI group demonstrated metabolic syndrome-related variables, including dyslipidemia.⁵ These data are consistent with the proposal that elevated fatty acid metabolism is higher in able bodied individuals with insulin resistance,^{29,30} but has not been previously reported in individuals with SCI. Although BMI did not differ significantly between the two groups, the highly significant decrease in LTM and the increase in total fat mass (total FM) in the SCI group provides evidence for increasing adiposity in this group. However, the significant increase in leptin in the SCI men indicates a lack of correlation between the adiposity and the leptin-lipid regulatory feedback loop;^{31,32} indicating that there is an overall disturbance in metabolic feedback control. No significant difference was identified for adiponectin.

Overall, the current results indicating disturbances with fatty acid biosynthesis in SCI individuals are consistent with the identification of increasing intramuscular fat, decreasing LTM and associated glucose intolerance.^{33,34} The increased leg FM in the SCI individuals provides the basis for the increased plasma leptin identified in the SCI, as leg fat mass has been previously shown to be a net leptin producer.^{35,36} We suggest that this source of leptin may link with the associated demineralization identified in SCI individuals given that elevated leptin levels have been previously reported to be associated with bone demineralization.^{37,38}

Evidence for the overall disturbance in fatty acid biosynthesis, in particular de-novo lipogenesis (C16:0/C18:2 ratio) and elongase (C18:0/C16:0 ratio) is not supported by the current data, although both fatty acid ratios were slightly higher in the SCI group. However, although the elongases have been implicated in diabetes, the overall pathophysiology of the elongase enzymes are not yet fully understood.^{11,39,40}

We acknowledge the limitations in this study, particularly related to the small sample size. However, despite only 20 participants in each group, all men in this study were carefully matched and as such, provide an

interesting view of this area, which has not, to date, been reported in persons with SCI. The different lesion levels may also be a limitation, although it is not unusual to find studies that group individuals with differing SCI levels for analyses. Furthermore, as this information is novel, there is no evidence to suggest that the degree of sympathetic denervation impacts fatty acid metabolism. The surrogates of fatty acid metabolism may not be a true and accurate reflection of enzyme activity; however, they have been used to describe associations with obesity, diabetes and metabolic syndrome risk by others.^{25,26} We did not collect dietary data in these groups, which may have been an issue given that individuals with SCI have been reported to consume a diet with higher fat content.⁴¹ In additional, physical activity levels were collected via self-report questionnaire, which are frequently associated with over- or under-estimation of activity (Supplemental material).

Conclusion

We have identified disturbances in fatty acid biosynthesis in the SCI group, which appears not to be fully regulated by feedback loops. Enzyme activity is associated with different tissue compartments and carbohydrate metabolism in SCI individuals and able-bodied controls. The loss of LTM and increase in FM with denervation in SCI is likely to disturb the normal skeletal muscle-fatty acid metabolic processes leading to the development of insulin resistance and ultimately metabolic syndrome.^{15,42} However, further work relating to the involvement of fatty acid metabolism and the related enzymatic pathways is necessary to fully understand the metabolic implications of dysfunctional lipogenesis in individuals with spinal cord injury.

Disclaimer statements

Contributors None.

Conflict of interest None.

Ethics approval None.

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References

- 1 Jones LM, Goulding A, Gerrard DF. DEXA: a practical and accurate tool to demonstrate total and regional bone loss, lean tissue loss and fat mass gain in paraplegia. *Spinal Cord* 1998;36(9): 637–40.
- 2 Jones LM, Legge M, Goulding A. Healthy body mass index (BMI) values often underestimate body fat in spinal cord injured males. *Arch Phys Med Rehabil* 2003;84(7):1068–71.

- 3 Bauman WA, Spungen AM. Carbohydrate and lipid metabolism in chronic spinal cord injury. *J Spinal Cord Med* 2001;24(4):266–77.
- 4 Frankel HL, Coll JR, Charlifue SW, Whiteneck GG, Gardner BP, Jamous MA, *et al.* Long-term survival in spinal cord injury: a fifty year investigation. *Spinal Cord* 1998;36(4):266–74.
- 5 Jones LM, Legge M, Goulding A. Factor analysis of the metabolic syndrome in spinal cord injured men. *Metabolism* 2004;53(10):1372–7.
- 6 Lee D-E, Kehlenbrink S, Lee H, Hawkins M, Yudkin JS. Getting the message across: mechanisms of physiological cross talk by adipose tissue. *Am J Physiol* 2009;296(6):E1210–29.
- 7 Jones LM, Legge M, Stoner L. Case-controlled, cross-sectional evidence that spinal cord injury associated with sympathetic decentralisation may alter osteocalcin signalling. *International Journal of Physical Medicine and Rehabilitation* 2014;2(224):doi:10.4172/2329-9096.1000224.
- 8 Avramoglu RK, Basciano H, Adeli K. Lipid and lipoprotein dysregulation in insulin resistance. *Clin Chim Acta* 2006;368(1–2):1–19.
- 9 Warensjö E, Öhrvall M, Vessby B. Fatty acid composition and estimated desaturase activities are associated with obesity and lifestyle variables in men and women. *Nutr Metab Cardiovasc Dis* 2006;16(2):128–36.
- 10 Vessby B. Dietary fat, fatty acid composition in plasma and the metabolic syndrome. *Curr Opin Lipidol* 2003;14(1):15–9.
- 11 Vessby B, Gustafsson IB, Tengblad S, Boberg M, Andersson A. Desaturation and elongation of fatty acids and insulin action. *Ann N Y Acad Sci* 2002;967:183–95.
- 12 Peter A, Cegan A, Wagner S, Lehmann R, Stefan N, Konigsrainer A, *et al.* Hepatic lipid composition and stearoyl-coenzyme A desaturase 1 mRNA expression can be estimated from plasma VLDL fatty acid ratios. *Clin Chem* 2009;55(12):2113–20.
- 13 Kroger J, Schulze MB. Recent insights into the relation of delta5 desaturase and delta6 desaturase activity to the development of type 2 diabetes. *Curr Opin Lipidol* 2012;23(1):4–10.
- 14 Gutierrez-Juarez R, Pocai A, Mulas C, Ono H, Bhanot S, Monia BP, *et al.* Critical role of stearoyl-CoA desaturase-1 (SCD1) in the onset of diet-induced hepatic insulin resistance. *J Clin Invest* 2006;116(6):1686–95.
- 15 Kraegen EW, Cooney GJ. Free fatty acids and skeletal muscle insulin resistance. *Curr Opin Lipidol* 2008;19(3):235–41.
- 16 Kirshblum SC, Burns SP, Biering-Sorensen F, Donovan W, Graves DE, Jha A, *et al.* International standards for neurological classification of spinal cord injury (Revised 2011). *J Spinal Cord Med* 2011;34:535–46.
- 17 Godin G, Shephard RJ. A simple method to assess exercise behavior in the community. *Can J Appl Sport Sci* 1985;10(3):141–6.
- 18 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28(7):412–9.
- 19 Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing. *Diabetes Care* 1999;22(9):1462–70.
- 20 Blish EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 1959;37(8):911–7.
- 21 Holub BJ, Skeaff CM. Nutritional regulation of cellular phosphatidylinositol. *Methods Enzymol* 1987;141:234–44.
- 22 Hodson L, Eyles HC, McLachlan KJ, Bell ML, Green TJ, Skeaff CM. Plasma and erythrocyte fatty acids reflect intakes of saturated and n-6 PUFA within a similar time frame. *J Nutr* 2014;144(1):33–41.
- 23 Cohen J. Statistical power for the behavioral sciences. 2nd ed. Hillsdale, NJ: Lawrence Erlbaum Associates; 1988.
- 24 Ntambi JM. Regulation of stearoyl-CoA desaturase by polyunsaturated fatty acids and cholesterol. *J Lipid Res* 1999;40(9):1549–58.
- 25 Warensjö E, Riserus U, Vessby B. Fatty acid composition of serum lipids predicts the development of the metabolic syndrome in men. *Diabetologia* 2005;48(10):1999–2005.
- 26 Warensjö E, Rosell M, Hellenius M-L, Vessby B, De Faire U, Riserus U. Associations between estimated fatty acid desaturase activities in serum lipids and adipose tissue in humans: links to obesity and insulin resistance. *Lipids Health Dis* 2009;8(37):doi:10.1186/476-511X-8-37.
- 27 Crofts CAP, Zinn C, Wheldon M, Schofield G. Hyperinsulinemia: A unifying theory of chronic disease? *Diabetes* 2015;1:34–43.
- 28 Hayashi T, Boyko EJ, Sato KK, McNeely MJ, Leonetti DL, Kahn SE, *et al.* Patterns of insulin concentration during the OGTT predict the risk of Type 2 diabetes in Japanese Americans. *Diabetes Care* 2013;36(5):1229–35.
- 29 Moore SC, Matthews CE, Sampson JN, Stolzenberg-Solomon RZ, Zheng W, Cai Q, *et al.* Human metabolic correlates of body mass index. *Metabolomics* 2014;10(2):259–69.
- 30 Newgard CB. Interplay between lipids and branched-chain amino acids in development of insulin resistance. *Cell Metab* 2012;15(5):606–14.
- 31 Cohen P, Friedman JM. Leptin and the control of metabolism: role of stearoyl-CoA desaturase-1 (SCD-1). *J Nutr* 2004;134(9):2455S–63S.
- 32 Mora S, Pessin JE. An adipocentric view of signaling and intracellular trafficking. *Diabetes Metab Res Rev* 2002;18(5):345–56.
- 33 Elder CP, Apple DF, Bickel CS, Meyer RA, Dudley GA. Intramuscular fat and glucose tolerance after spinal cord injury--a cross-sectional study. *Spinal Cord* 2004;42:711–6.
- 34 Gorgey AS, Dudley GA. Skeletal muscle atrophy and increased intramuscular fat after incomplete spinal cord injury. *Spinal Cord* 2006;45:304–9.
- 35 Jensen MD, Möller N, Sreekumaran Nair K, Eisenberg P, Landt M, Klein S. Regional leptin kinetics in humans. *Am J Clin Nutr* 1999;69:18–21.
- 36 Manolopoulos KN, Karpe F, Frayn KN. Gluteofemoral body fat as a determinant of metabolic health. *Int J Obes* 2010;34:949–59.
- 37 Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab* 2004;89:2548–56.
- 38 Sato M, Takeda N, Sarui H, Takami R, Takami K, Hayashi M, *et al.* Association between serum leptin concentrations and bone mineral density, and biochemical markers of bone turnover in adult men. *J Clin Endocrinol Metab* 2001;86:5273–6.
- 39 Jakobsson A, Westerberg R, Jakobsson A. Fatty acid elongases in mammals: their regulation and roles in metabolism. *Prog Lipid Res* 2006;45(3):237–49.
- 40 Leonard AE, Pereira SL, Sprecher H, Huang YS. Elongation of long-chain fatty acids. *Prog Lipid Res* 2004;43(1):36–54.
- 41 Groah SL, Nash MS, Ljungberg IH, Libin A, Hamm LF, Ward E, *et al.* Nutrient intake and body habitus after spinal cord injury: an analysis by sex and level of injury. *J Spinal Cord Med* 2009;32(1):25–33.
- 42 Silveira LR, Fiamoncini J, Hirabara SM, Procopio J, Cambiaghi TD, Pinheiro CH, *et al.* Updating the effects of fatty acids on skeletal muscle. *J Cell Physiol* 2008;217(1):1–12.