Lipid Metabolism, Abdominal Adiposity, and Cerebral Health in the Amish

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Objective: To assess the association between peripheral lipid/fat profiles and cerebral gray matter (GM) and white matter (WM) in healthy Old Order Amish (OOA).

Methods: Blood lipids, abdominal adiposity, liver lipid contents, and cerebral microstructure were assessed in OOA (N = 64, 31 males/33 females, ages 18-77). Orthogonal factors were extracted from lipid and imaging adiposity measures. GM assessment used the Human Connectome Project protocol to measure whole-brain average cortical thickness. Diffusion-weighted imaging was used to derive WM fractional anisotropy and kurtosis anisotropy measurements.

Results: Lipid/fat measures were captured by three orthogonal factors explaining 80% of the variance. Factor one loaded on cholesterol and/or low-density lipoprotein cholesterol measurements; factor two loaded on triglyceride/liver measurements; and factor three loaded on abdominal fat measurements. A two-stage regression including age/sex (first stage) and the three factors (second stage) examined the peripheral lipid/fat effects. Factors two and three significantly contributed to WM measures after Bonferroni corrections (P < 0.007). No factor significantly contributed to GM. Blood pressure (BP) inclusion did not meaningfully alter the lipid/fat-WM relationship.

Conclusions: Peripheral lipid/fat indicators were significantly and negatively associated with cerebral WM rather than with GM, independent of age and BP level. Dissecting the fat/lipid components contributing to different brain imaging parameters may open a new understanding of the body-brain connection through lipid metabolism.

Introduction

Adiposity underlies energy storage, hormone regulation, and other important functions (1). Excessive adiposity is a risk factor for reduced cerebral integrity (2). We systematically evaluated body adiposity and blood lipids as potential risk factors for brain microstructure in a sample of healthy and largely normotensive Old Order Amish (OOA). Compared to the general population, OOA have uniform farm-dwelling lifestyles and relative genetic and environmental homogeneity (see Supporting Information) (3). This cohort also has a relatively uniform diet and greatly reduces potential confounds due to alcohol, tobacco, and illicit substance use (3), thus constituting a good sample for studying body-brain relationships.

We combined advanced body imaging and liver spectroscopy with standard clinical lipid assessment to derive lipid and adiposity profiles. We utilized high-resolution structural and diffusion kurtosis imaging (DKI) to assess cerebral integrity. DKI is a model-independent extension of diffusion tensor imaging that accounts for non-Gaussian diffusion observed in cerebral white matter (WM) and gray matter (GM) (4). DKI calculates diffusion tensor imaging’s fractional anisotropy (FA), axial diffusivity and/or radial diffusivity (RD) parameters, axial kurtosis (AK) and/or radial kurtosis (RK), and kurtosis anisotropy (KA) to capture non-Gaussian diffusion behavior of diffusing water molecules. These measures show higher sensitivity to tissue integrity impairment in stroke and schizophrenia (5,6) (see Supporting Information).
We hypothesized that cerebral WM may be particularly sensitive to increased adiposity/lipids, even under healthy conditions, because of high lipiddensity in myelinated WM and previously established susceptibility to cardiovascular/metabolic factors (7,8) and adiposity (9).

Methods

Subjects

The participants were from the Amish Connectome Project (N = 64, 31 males/33 females; ages 18-77 years, average age 46.3 ± 17.5 years). Subjects underwent a medical assessment and lipid panel analysis (Table 1; see also Supporting Information). Every subject signed a written informed consent form approved by the University of Maryland Institutional Review Board.

Fat and brain imaging

Magnetic resonance imaging examination consisted of high-resolution abdominal fat imaging, magnetic resonance spectroscopy measurement of hepatic fat concentration, and diffusion-weighted brain imaging using a 3T Siemens Trio scanner (Siemens Medical Solutions, Inc., Malvern, Pennsylvania) with a 32-channel head coil and an eight-channel body coil at the Maryland Psychiatric Research Center (see Supporting Information). DKI analysis extracted whole-brain averaged FA, KA, axial diffusivity and RD, and AK and RK.

Statistical analyses

We identified peripheral lipid/adiposity profile factors by entering blood and imaging-based data into a principal component analysis to uncover related measures, reduce the number of dependent variables, and minimize colinearity. A varimax rotation orthogonalized the individual eigenvectors.

A general linear mixed effect model used the lipid/fat factors as predictors for averaged whole-brain GM cortical thickness and WM metrics in separate general linear mixed effect models (see Supporting Information). Bonferroni correction set significance to P < 0.007.

| TABLE 1 | Demographic information for study participants |
|---------|-------------------------------------------|
|         | **Average** | **Range** |
| **Age, y** | 46.3 ± 17.5 | 18-77 |
| **Height, cm** | 168.27 ± 9.39 | 149.4-184.8 |
| **Weight, kg** | 83.19 ± 12.85 | 51.2-109.6 |
| **BMI (% of sample with underweight, normal weight, overweight, or obesity)** | 28.16 ± 5.20 (1.6, 37.5, 23.4, 37.5) | 17.66-39.01 |
| **Systolic BP, mm Hg (% of sample > 140 mm Hg)** | 120.79 ± 15.25 (9.5) | 94-169 |
| **Diastolic BP, mm Hg (% of sample > 90 mm Hg)** | 71.23 ± 7.99 (4.8) | 56-91 |
| **Cholesterol, mg/dL (% of sample with borderline, high)** | 209.8 ± 43.6 (39.06, 25.00) | 108-315 |
| **LDL-C, mg/dL (% of sample with borderline, high, very high)** | 131.5 ± 38.4 (35.95, 12.50, 7.94) | 51-246 |
| **Triglycerides, mg/dL (% of sample with borderline, high)** | 86.5 ± 45.66 (12.50, 1.56) | 34-264 |

BMI classified as follows: underweight, < 18.5; normal, 18.5-24.9; overweight, 25-29.9; and obesity, > 30. Blood cholesterol levels classified as follows, high, > 240 mg/dL; and borderline, 200-239 mg/dL. Blood LDL-C levels classified as follows: borderline high, 130-159 mg/dL; high, 160-189 mg/dL; and very high, > 190 mg/dL. Blood triglyceride levels classified as follows: borderline, 150-199 mg/dL and high, 200-499 mg/dL.

Results

Factor analysis

Three orthogonal factors captured 80% of the total variance (Supporting Information Table S1). Factor one loaded with total cholesterol and low-density lipoprotein cholesterol (LDL-C); factor two loaded with blood triglyceride, liver fat concentration, and high-density lipoprotein cholesterol; and factor three loaded with abdominal and perirenal fat volumes. The factors were relabeled as “cholesterol factor (CF),” “triglyceride factor (TF),” and “abdominal fat factor (AFF)” for factors one, two, and three, respectively.

**WM microstructure**

The model was significant for whole-brain WM average FA (χ² = 25.9, P = 9.3-10⁻⁵), KA (χ² = 50.3, P = 1-10⁻¹⁰), RD (χ² = 18.7, P = 0.003), AK (χ² = 37.7, P = 4.10⁻¹⁰), and RK (χ² = 31.4, P = 8.10⁻⁶) (Table 2).

After considering age, sex, and household covariates, the CF showed no significant WM associations. The TF showed significantly positive associations with RD (βTF = 6.4 ± 2.10⁻⁵, P = 0.002) and AK (βTF = 1.2 ± 0.410⁻², P = 0.001). The AFF showed a significantly negative KA association (βAFF = −4.7 ± 1.710⁻¹⁰, P = 0.004) and a significantly positive AK association (βAFF = 2.5 ± 0.610⁻², P = 2.10⁻⁵) (Table 2).

Repeat analysis included BMI, which proved to be an insignificant predictor (Supporting Information Table S2).

**GM microstructure**

The model was significant for whole-brain average cortical GM thickness (χ² = 55.3, P = 1-10⁻¹²). After considering age, sex, and household covariates, average GM thickness showed no significant association with any factor (Table 2). Experimental DKI showed no significant factor associations (Supporting Information Table S3).

**Potential blood pressure effects**

Blood pressure (BP) showed no significant association with whole-brain measurements, TF, or CF (P > 0.3). BP was significantly and positively correlated with the AFF (P < 0.001). Lipid/fat-WM
associations were repeated to include systolic and diastolic BP, which rendered the TF-RD relationship insignificant (Table 3). The TF-AK, AFF-KA, and AFF-AK relationships remained statistically significant. Lipid/fat and whole-brain GM cortical thickness relationships remained insignificant (Table 3).

**Discussion**

We reported lipid/fat profiles and cerebral WM associations in healthy and mainly normotensive OOA. We showed that increases in abdominal fat, liver adiposity, and circulating triglyceride levels negatively correlated with cerebral WM integrity beyond hypertension. This suggested that abdominal adiposity and/or high blood lipids may present cerebral risk, even in physically active and normotensive individuals, but whether the WM-specific relationship is causal or secondary remains unclear. The associations were not significant for cerebral GM, potentially because of differences in cerebrovascular architecture between cortical GM and WM, in which perfusion rate in cerebral WM is lower (10). Additionally, WM has been shown to be particularly sensitive to systemic inflammation and obesity-associated metabolic disorders (5-8).

Adiposity imaging and spectroscopy formed three orthogonal factors. Total cholesterol and LDL-C formed the CF, which is consistent with clinical implications of elevated total cholesterol and LDL-C as risk factors for ischemic heart disease (11). Triglycerides, liver fat fractions, and high-density lipoprotein cholesterol formed the TF. Triglycerides are primarily synthesized and/or stored in the liver, and triglyceridermia is a risk factor for nonalcoholic fatty liver disease (12). The AFF was based on abdominal and perirenal fat volumes. Abdominal obesity has been shown to be directly associated with atherosclerosis progression (13), and perirenal fat may have specific cardiovascular risks (14). Overall, there is confidence in the biological validity of the factors.

Previous imaging studies in aging or hypertensive populations have reported negative associations between abdominal obesity and cerebral WM FA (15,16). The obesity-WM relationship in hypertensive subjects has been interpreted as driven by BP and secondary to obesity (15). Although not statistically significant after Bonferroni correction, our normotensive sample replicated the negative FA-abdominal obesity association ($r = -0.45, P = 0.04$) (15,16), which became marginally stronger with BP as covariates ($P = 0.02$, Table 3). Therefore, abdominal adiposity may associate with FA beyond a BP mechanism. The AFF was significantly and negatively associated with KA, driven by AK (see Supporting Information). Elevation in AK has been observed in stroke and brain trauma, reflecting inflammation-related changes in the intra-axonal space (6). Higher AK values have also been observed in neuropsychiatric conditions, reflecting neuroinflammation (5).

The underlying mechanisms of the AFF-WM associations are complex. Obesity is a risk factor for hypertension that directly affects cerebral integrity (7,8,17) due to the stenosis of long-penetrating cerebral blood vessels that perfuse cerebral WM (7). Post hoc analysis showed a negative correlation between systolic BP and FA ($r = -0.34, P = 0.004$) (Supporting Information Figure S1). However, including BP did not meaningfully alter the lipid/fat factor-WM relationships (Tables 3), which suggests that, in normotensive individuals, abdominal adiposity may correlate with WM through a yet-to-be-determined mechanism. A recent study formed a similar conclusion, stating that adiposity-WM associations partially resulted from mechanisms other than BP (18).

TF scores were associated with WM AK ($P = 0.001$), even after inclusion of BP (see Supporting Information). The TF-RD
| Subcortical WM | $\beta_{\text{age}} (P)$  | $B_{\text{sex}} (P)$  | $\beta_{\text{CF}} (P)$  | $\beta_{\text{TF}} (P)$  | $\beta_{\text{AFF}} (P)$  | $\beta_{\text{SBP}} (P)$  | $\beta_{\text{DBP}} (P)$  | Model, $\chi^2 (P)$ |
|----------------|-----------------|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| FA             | -3.27E-04 ± 1.37E-04 | -1.45E-03 ± 4.36E-03 | -8.12E-04 ± 1.96E-03 | 1.58E-03 ± 1.64E-03 | -5.75E-03 ± 2.59E-03 | -1.60E-04 ± 1.36E-04 | -4.55E-04 ± 2.83E-04 | 29.14 |
|               | (0.01)           | (0.72)          | (0.66)        | (0.30)        | (0.02)         | (0.21)         | (0.09)         | (1.4E-04)     |
| KA             | -4.39E-04 ± 9.73E-05 | 8.73E-04 ± 3.10E-03 | -1.30E-03 ± 1.29E-03 | -1.09E-03 ± 1.18E-03 | -6.16E-03 ± 1.84E-03 | 7.20E-05 ± 9.81E-05 | -2.90E-04 ± 2.00E-04 | 54.91 |
|               | (8.40E-06)       | (0.01)          | (0.28)        | (0.32)        | (8.31E-04)     | (0.43)         | (0.13)         | (1.6E-09)     |
| AD             | -8.69E-06 ± 5.32E-06 | -4.72E-06 ± 1.53E-04 | 1.33E-04 ± 6.75E-05 | 1.32E-04 ± 6.35E-05 | 2.30E-05 ± 9.45E-05 | -3.21E-08 ± 4.98E-06 | -4.96E-06 ± 9.97E-06 | 9.56 |
|               | (0.11)           | (0.76)          | (0.06)        | (0.04)        | (9.81)         | (0.99)         | (0.03)         | (0.21)        |
| RD             | -2.52E-06 ± 1.84E-06 | -9.31E-06 ± 5.06E-05 | 5.30E-05 ± 2.39E-05 | 4.08E-05 ± 2.23E-05 | 1.88E-05 ± 3.39E-05 | 2.86E-07 ± 1.80E-06 | 1.04E-06 ± 3.67E-06 | 9.32 |
|               | (0.21)           | (0.83)          | (0.02)        | (0.04)        | (0.53)         | (0.89)         | (0.82)         | (0.23)        |
| AK             | 1.81E-04 ± 3.23E-04 | 1.70E-03 ± 1.00E-02 | 6.84E-03 ± 4.22E-03 | 1.27E-02 ± 3.92E-03 | 2.79E-02 ± 6.02E-03 | -4.39E-04 ± 3.21E-04 | -5.23E-04 ± 6.57E-04 | 41.98 |
|               | (0.60)           | (0.86)          | (0.09)        | (8.83E-04)    | (5.66E-06)     | (0.17)         | (0.39)         | (5.2E-07)     |
| RK             | -2.69E-03 ± 6.20E-04 | -4.25E-03 ± 1.97E-02 | 1.14E-02 ± 8.19E-03 | -2.11E-04 ± 7.53E-03 | -1.23E-02 ± 1.17E-02 | -7.80E-04 ± 6.26E-04 | -2.73E-03 ± 1.29E-03 | 36.46 |
|               | (1.82E-05)       | (0.82)          | (0.14)        | (0.98)        | (0.26)         | (0.18)         | (0.03)         | (5.9E-06)     |
| Cortical GM    | -9.13E-03 ± 1.85E-03 | 4.56E-02 ± 5.75E-02 | -2.83E-02 ± 2.49E-02 | 1.69E-02 ± 2.51E-02 | 2.22E-02 ± 3.37E-02 | -3.06E-03 ± 1.87E-03 | 1.10E-04 ± 4.00E-03 | 44.49 |
|                | (4.03E-06)       | (0.37)          | (0.24)        | (0.48)        | (0.45)         | (0.09)         | (0.99)         | (1.7E-07)     |

Bolded values indicate statistical significance after correction for seven regression analyses ($P < 0.05/7 = 0.007$). Italicized values indicate suggestive significance ($0.05 < P < 0.007$).

AD = axial diffusivity; DBP = diastolic BP; SBP = systolic BP; CF = cholesterol factor; TF = triglyceride factor; AFF = abdominal fat factor; FA = fractional anisotropy; KA, kurtosis anisotropy; RD = radial diffusivity; AK = axial kurtosis; RK = radial kurtosis; WM = white matter; GM = gray matter.
relationship was rendered insignificant after BP inclusion, partly because RD was significantly associated with systolic ($r = 0.33$) and diastolic ($r = 0.25$) BP. Therefore, BP may drive the TF-RD relationship. A study in normotensive and prehypertensive adults associated BP with RD, concluding that increased adiposity affects WM directly and indirectly through BP mediated pathways (18).

We replicated the significant age-associated reduction in cortical GM thickness. However, diffusion-weighted magnetic resonance imaging parameters showed no significant factor associations. These results are considered experimental (Supporting Information Table S3), but they suggest that increased body lipids and fat have less association with GM, potentially because of a higher lipid content in myelinated WM and cerebrovascular architectural differences (7,8). Therefore, WM may be more sensitive to fat/lipid factors than GM.

The CF was not significantly correlated with GM or WM. Williams and colleagues (19) observed a negative FA-LDL-C association in older adults, which they postulated as a risk for Alzheimer’s and vascular dementias caused by elevated BP and atherosclerosis. Directly comparing results is difficult because the sample used by Williams was older, with 30% of the subjects using cholesterol-controlling medications (19). Moreover, total cholesterol and LDL-C in OOA were not significantly correlated with their suggested BP mediation mechanism ($r < 0.05, P > 0.5$) (19). Instead, our study showed that BP might present more subtly in a healthy population. Both studies reported significantly negative triglyceride-WM associations.

Specific biological interpretations are limited, as diffusion metrics are mathematically derived and as underlying neurobiological correlates are not fully understood (20). This study also used a small number of subjects ($N = 64$), which prevented detailed causal exploration of WM-adiposity relationships. Further statistical analysis did not show significant gender contrasts for WM-adiposity relationships. Males and females did not differ on BMI, WM, or GM measurements (all $P > 0.4$) after age correction. The OOA participants may be considered both an advantage and a study limitation. The clear advantage in our study was the environmental uniformity and minimal confounds associated with the participants. However, the generalizability to the US population may be limited. OOA were primarily normotensive (<10% hypertensive) compared to the US population (~30%) but comparable in BMI. If the primary goal is to understand peripheral lipid/fat-brain integrity relationships, OOA are an excellent cohort to examine this question under healthy conditions. Therefore, we believe the advantages outweigh potential generalizability limitations.

Conclusion

We performed a comprehensive analysis on the associations between lipids/adiposity and cerebral integrity in healthy OOA. The multifactorial analysis demonstrated that increases in abdominal fat, liver adiposity, and circulating triglycerides significantly associate with cerebral WM microstructure. The relationship with cerebral GM was much less obvious.

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References

1. Cohen P, Spiegelman BM. Cell biology of fat storage. *Med Biol Cell* 2016;27:2523-2527.
2. Marks BL, Katz LM, Styner M, Smith JK. Aerobic fitness and obesity: relationship to cerebral white matter integrity in the brain of active and sedentary older adults. *Br J Sports Med* 2010;45:1206-1215.
3. Nugent KL, Million-Mekva A, Backman J, et al. Familial aggregation of tobacco use behaviors in Amish men. *Nicotine Tob Res* 2013;16:923-930.
4. Jensen JH, Helpern JA, Raman J, Alu, Luczkynski K. Diffusional kurtosis imaging: the quantification of non-gaussian water diffusion by means of magnetic resonance imaging. *Magn Reson Med* 2003;53:1432-1440.
5. Kochunov P, Rowland LM, Fieremans E, et al. Diffusion-weighted imaging uncovers likely sources of processing-speed deficits in schizophrenia. *Proc Natl Acad Sci U S A* 2016;113:13504-13509.
6. Weber RA, Hui ES, Jensen JH, et al. Diffusional kurtosis and diffusion tensor imaging reveal different time-sensitive stroke-induced microstructural changes. *Stroke* 2016;46:545-550.
7. Kochunov P, Glahn D, Lancaster J, et al. Whole brain and regional hyperintense white matter volume and blood pressure: overlap of genetic loci produced by bivariate, whole-genome linkage analyses. *Stroke* 2010;41:2137-2142.
8. Kochunov P, Glahn DC, Lancaster J, et al. Blood pressure and cerebral white matter share common genetic factors in Mexican Americans. *Hypertension* 2011;57:330-335.
9. Jagust W, Harvey D, Mungas D, Haan M. Central obesity and the aging brain. *Arch Neurol* 2005;62:1545-1548.
10. Wright SN, Kochunov P, Chiappelli J, et al. Accelerated white matter aging in schizophrenia: role of white matter blood perfusion. *Neurol Biol Aging* 2014;35:2411-2418.
11. Prospective Studies Collaboration; Lewington S, Whitley C, Clark R, et al. Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. *Lancet* 2007;370:1829-1839.
12. Ramasamy I. Recent advances in physiological lipoprotein metabolism. *Clin Chem Lab Med* 2014;52:1695-1727.
13. Després JP, Moeran J, Lupien PJ, Tremblay A, Nadeau A, Bouchard C. Regional distribution of body fat, plasma lipoproteins, and cardiovascular disease. *Arteriosclerosis* 1990;10:497-511.
14. De Pergola G, Campobasso N, Nardecchia A, et al., Para- and perirenal ultrasonographic fat thickness is associated with 24-hours mean diastolic blood pressure levels in overweight and obese subjects. *BMC Cardiovasc Disord* 2015;15:108. doi:10.1186/s12872-015-0101-6
15. Spicker EA, Kochunov P, Rowland LM, et al. Shared genetic variance between obesity and white matter integrity in Mexican Americans. *Front Genet* 2015;6:26. doi:10.3389/fgene.2015.00026
16. Kullmann S, Callaghan MF, Henri M, et al. Specific white matter tissue microstructure changes associated with obesity. *Neuroimage* 2016;125:36-44.
17. Kochunov P, Glahn DC, Hong LE, et al. P-selectin expression tracks cerebral atrophy in Mexican-Americans. *Front Genet* 2012;3:65. doi:10.3389/fgene.2012.00065
18. Allen B, Muldowny MF, Gianaros PJ, Jennings JR. Higher blood pressure partially links greater adiposity to reduced brain white matter integrity. *Am J Hypertens* 2016;29:1029-1037.
19. Williams VJ, Lertiz EC, Shepel J, et al. Interindividual variation in serum cholesterol is associated with regional white matter tissue integrity in older adults. *Hum Brain Mapp* 2013;34:1826-1841.
20. Grinberg F, Furrer E, Kaffanke J, Oros-Pequens AM, Shah NJ. Non-Gaussian diffusion in human brain tissue at high b-factors as examined by a combined diffusion kurtosis and biexponential diffusion tensor analysis. *Neuroimage* 2011;57:1087-1102.