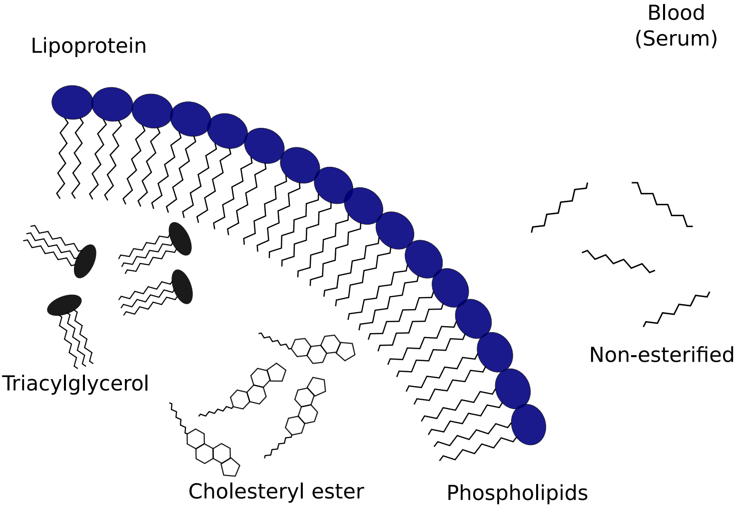
Fatty acid composition in four serum lipid fractions and the pathogenesis of diabetes

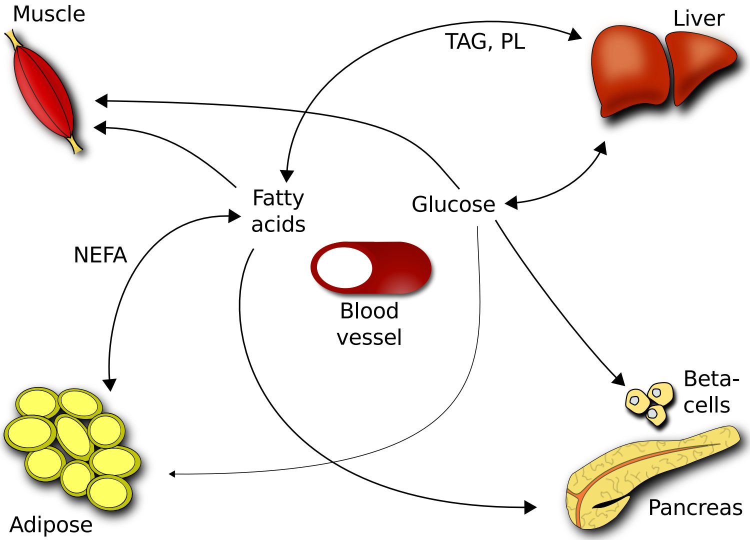
Luke Johnston

Grand Finale (4th)
Oct. 27th, 2016

Physiology of serum lipid fractions



Glucose and fatty acid metabolism



Various fatty acid length and desaturation

- Range in length and number of double bonds
- Fatty acids either from diet or *de novo* lipogenesis (DNL)
- Physiological role dependent on molecule
- Eg: higher palmitic acid (16:0) lipotoxic to beta-cells *in vivo* and *in vitro*[1]

[1] Giacca et al. (2011); Xiao, Giacca, and Lewis (2009)

Few large cohorts on fatty acid composition, fraction, and diabetes

- One study (METSIM) had three fractions: TAG, PL, CE[2]
  - Multiple flaws
- Mainly cohorts report on PL and CE: CHS, EPIC, ARIC[3]
  - 16:0 and 18:0 higher risk for DM
  - 18:1n-7, 18:1n-9, 18:3n-3 lower risk for DM

---

[2] Lankinen et al. (2015)

[3] L. Wang et al. (2003); Forouhi et al. (2014); Kröger et al. (2011); Ma et al. (2015); Djoussé et al. (2011)

Objectives:

Explore associations of fatty acid composition of serum lipid fractions on diabetes pathogenesis:

Objectives:

Explore associations of fatty acid composition of serum lipid fractions on diabetes pathogenesis:

- NEFA: *Higher total NEFA, not individual fatty acids, contribute to lower beta-cell function*

Objectives:

Explore associations of fatty acid composition of serum lipid fractions on diabetes pathogenesis:

- NEFA: *Higher total NEFA, not individual fatty acids, contribute to lower beta-cell function*
- PL: *Higher palmitic acid associates with* ***declines*** *in beta-cell function over time. Higher cis-vaccenic acid associated with higher insulin sensitivity and beta-cell function.*

Objectives:

Explore associations of fatty acid composition of serum lipid fractions on diabetes pathogenesis:

- NEFA: *Higher total NEFA, not individual fatty acids, contribute to lower beta-cell function*
- PL: *Higher palmitic acid associates with* ***declines*** *in beta-cell function over time. Higher cis-vaccenic acid associated with higher insulin sensitivity and beta-cell function.*
- CE: *No strong associates with diabetes pathogenesis*

Objectives:

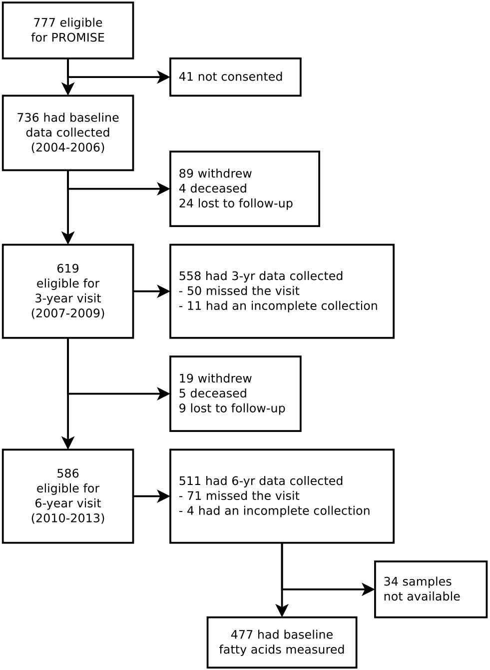
Explore associations of fatty acid composition of serum lipid fractions on diabetes pathogenesis:

- NEFA: *Higher total NEFA, not individual fatty acids, contribute to lower beta-cell function*
- PL: *Higher palmitic acid associates with* ***declines*** *in beta-cell function over time. Higher cis-vaccenic acid associated with higher insulin sensitivity and beta-cell function.*
- CE: *No strong associates with diabetes pathogenesis*
- TAG: …

Data source: The PROMISE cohort

PROspective Metabolism and ISlet cell Evaluation cohort.

- Recruited from London and Toronto centers
- Followed every ~3 years (3 time points completed)
- Demographics, lifestyle, anthropometrics, and blood


777 eligible for PROMISE
41 not consented
736 had baseline data collected (2004-2006)
89 withdrew
4 deceased
24 lost to follow-up
619 eligible for 3-year visit (2007-2009)
558 had 3-yr data collected
- 50 missed the visit
- 11 had an incomplete collection
19 withdrew
5 deceased
9 lost to follow-up
586 eligible for 6-year visit (2010-2013)
511 had 6-yr data collected
- 71 missed the visit
- 4 had an incomplete collection
34 samples not available
477 had baseline fatty acids measured

Variables of interest

Metabolic outcomes

Calculated from OGTT:

- Insulin sensitivity: 1/HOMA-IR, ISI
- Beta-cell function: IGI/HOMA-IR, ISSI-2

Variables of interest

Metabolic outcomes

Calculated from OGTT:

- Insulin sensitivity: 1/HOMA-IR, ISI
- Beta-cell function: IGI/HOMA-IR, ISSI-2

Median declines of 14% to 27%

Variables of interest

Metabolic outcomes

Calculated from OGTT:

- Insulin sensitivity: 1/HOMA-IR, ISI
- Beta-cell function: IGI/HOMA-IR, ISSI-2

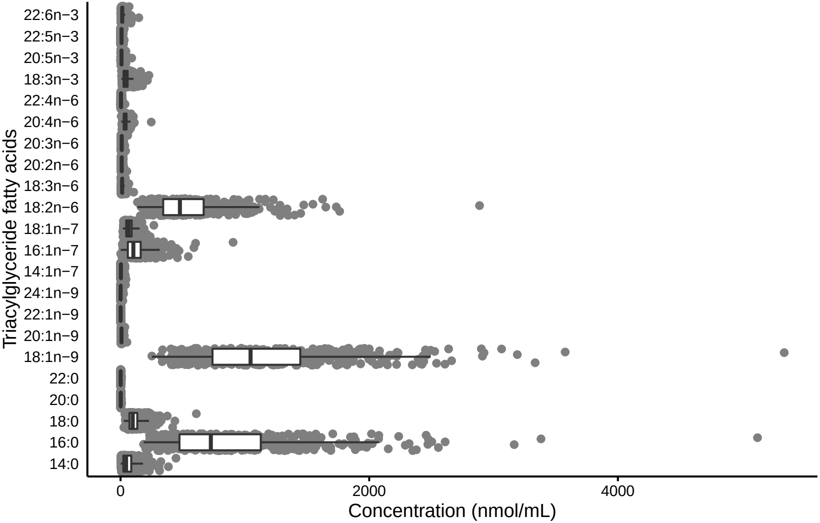
Median declines of 14% to 27%

TAG fatty acids

Thin layer chromatography to split the lipid fractions, gas chromatography for the fatty acids:

- 22 TAG fatty acids, as concentration (nmol/mL) and percent of total (mol%)

TAG fatty acid composition within PROMISE



Statistical analysis

Statistical analysis

R code for these results:

`https://github.com/lwjohnst86/seminar2016`

Statistical analysis: Generalized estimating equations (GEE)

Variables GEE model:

> *Visit number, waist size, baseline age, ethnicity, sex, ALT (marker of liver fat), physical activity (MET), and total NEFA.*

Time-*independent*: TAG, NEFA, baseline age, ethnicity, sex

Statistical analysis: Generalized estimating equations (GEE)

Variables GEE model:

> *Visit number, waist size, baseline age, ethnicity, sex, ALT (marker of liver fat), physical activity (MET), and total NEFA.*

Time-*independent*: TAG, NEFA, baseline age, ethnicity, sex

- Concern: multiple models will be computed

Statistical analysis: Generalized estimating equations (GEE)

Variables GEE model:

> *Visit number, waist size, baseline age, ethnicity, sex, ALT (marker of liver fat), physical activity (MET), and total NEFA.*

Time-*independent*: TAG, NEFA, baseline age, ethnicity, sex

- Concern: multiple models will be computed
- P-values: generally unreliable, especially with more tests[4]

---

[4] See the American Statistical Association statement on it

Statistical analysis: Generalized estimating equations (GEE)

Variables GEE model:

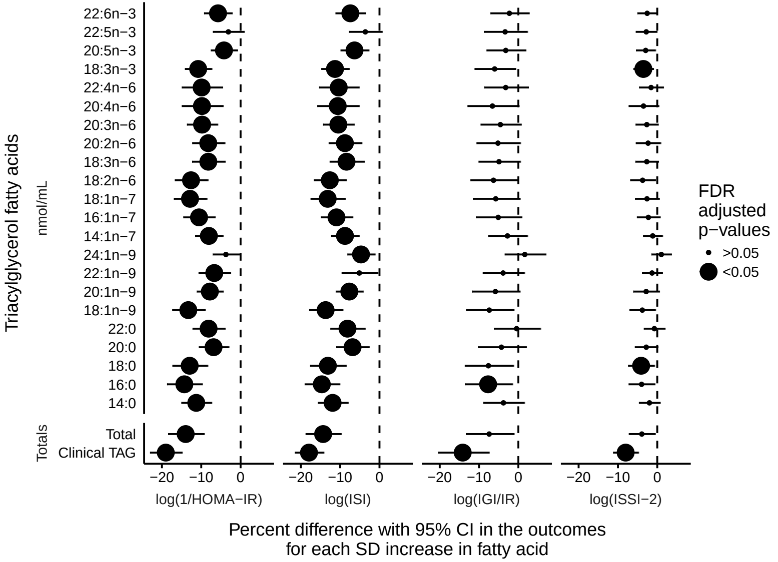
> *Visit number, waist size, baseline age, ethnicity, sex, ALT (marker of liver fat), physical activity (MET), and total NEFA.*

Time-*independent*: TAG, NEFA, baseline age, ethnicity, sex

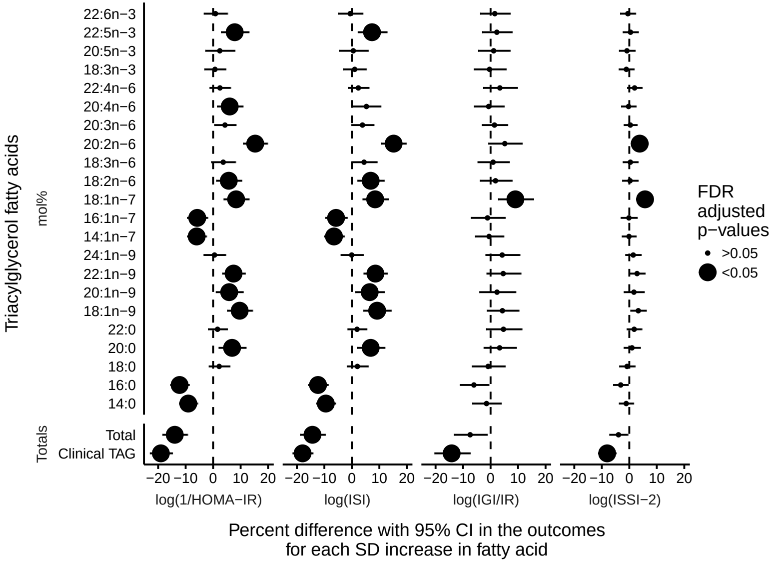
- Concern: multiple models will be computed
- P-values: generally unreliable, especially with more tests[4]
- Adjust using BH False Discovery Rate (FDR) correction

[4] See the American Statistical Association statement on it

As conc, strong negative association with IS (96 non-FDR vs 77 FDR of 184 models)



As mol%, very different story — different FA have positive or negative roles

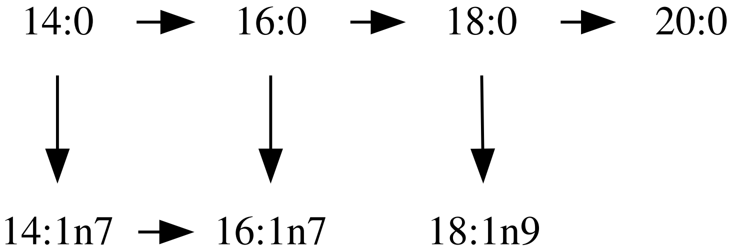


But... GEE modeling is limited

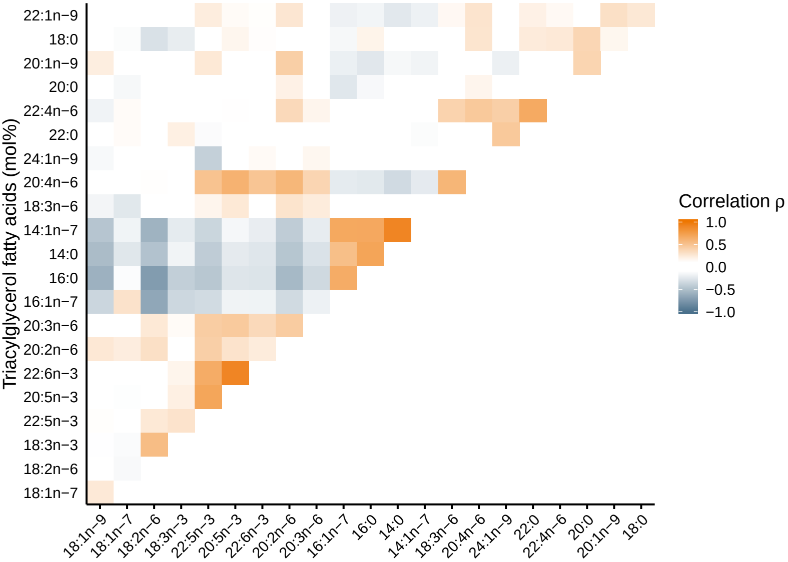
- TAG fatty acid composition in inherently multivariate

But… GEE modeling is limited

- TAG fatty acid composition in inherently multivariate



Correlation between TAG fatty acids



Partial Least Squares (PLS) allows for multivariate data

Takes:

$$ISI = 140 + 141n7 + ... + 225n3$$

Converts to:

$$ISI = Comp1 + Comp2$$

Partial Least Squares (PLS) allows for multivariate data

Takes:

$$ISI = 140 + 141n7 + ... + 225n3$$

Converts to:

$$ISI = Comp1 + Comp2$$

- PLS: No p-value, no p-value problem

Partial Least Squares (PLS) allows for multivariate data

Takes:

$$ISI = 140 + 141n7 + ... + 225n3$$

Converts to:

$$ISI = Comp1 + Comp2$$

- PLS: No p-value, no p-value problem
- Cross-validation (CV) determines predictability

Partial Least Squares (PLS) allows for multivariate data

Takes:

$$ISI = 140 + 141n7 + \ldots + 225n3$$

Converts to:

$$ISI = Comp1 + Comp2$$

- PLS: No p-value, no p-value problem
- Cross-validation (CV) determines predictability
- CV randomly splits data into training and test sets

Partial Least Squares (PLS) allows for multivariate data

Takes:

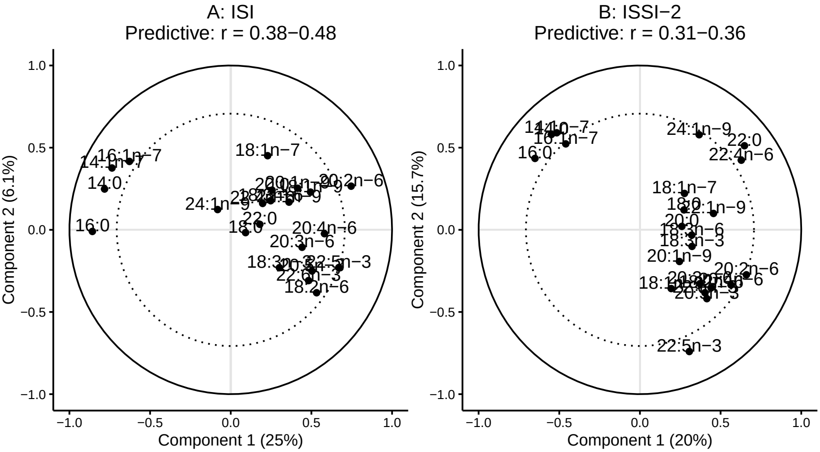
$$ISI = 140 + 141n7 + ... + 225n3$$

Converts to:

$$ISI = Comp1 + Comp2$$

- PLS: No p-value, no p-value problem
- Cross-validation (CV) determines predictability
- CV randomly splits data into training and test sets
- Limitation: Can only use one time point (cross-sectional) and no covariates

Four long chain fatty acids (14:0, 14:1n-7, 16:0, 16:1n-7) cluster and strongly explain the variance in metabolic function



FA involved in DNL from higher carb intake associate with lower metabolic functioning

- Upregulated DNL, increased 14 and 16 chain fatty acids[5]
  - 16:1n-7 shown to be highly related to directly measured DNL
  - Shown to be lipotoxic

[5] Lee et al. (2015); Wilke et al. (2009)
[6] Rhee et al. (2011); Lankinen et al. (2015)

FA involved in DNL from higher carb intake associate with lower metabolic functioning

- Upregulated DNL, increased 14 and 16 chain fatty acids[5]
  - 16:1n-7 shown to be highly related to directly measured DNL
  - Shown to be lipotoxic

- Two other cohort studies[6] had similar findings for diabetes and HOMA-IR.

[5] Lee et al. (2015); Wilke et al. (2009)
[6] Rhee et al. (2011); Lankinen et al. (2015)

Overall conclusions of PhD research

- Each lipid fraction behaves slightly differently on metabolic functioning
- Fatty acids from DNL may contribute to metabolic dysfunction
- Potential biomarker of DNL fatty acids for clinical use

Overall conclusions of PhD research

- Each lipid fraction behaves slightly differently on metabolic functioning
- Fatty acids from DNL may contribute to metabolic dysfunction
- Potential biomarker of DNL fatty acids for clinical use

- ...Make use of statistical and analytical advances

Acknowledgements

- **Supervisor:** Dr. Anthony Hanley
- **Co-Supervisor:** Dr. Richard Bazinet
- **Committee Member:** Dr. Adria Giacca
- **Hanley Lab:** Ingrid Santaren, Zhila Semnani-Azad, Windy Wang
- **Research Nurses:** Jan Neuman, Paula Van Nostrand, Stella Kink, Annette Barnie, Sheila Porter, Mauricio Marin
- **Funding:** CDA, CIHR, BBDC

Code: `https://github.com/lwjohnst86/seminar2016`

Canadian Diabetes Association

CIHR IRSC
Canadian Institutes of Health Research
Instituts de recherche en santé du Canada

Banting & Best Diabetes Centre
UNIVERSITY OF TORONTO

References

Djoussé, Luc, Mary L Biggs, Rozenn N Lemaitre, Irena B King, Xiaoling Song, Joachim H Ix, Kenneth J Mukamal, David S Siscovick, and Dariush Mozaffarian. 2011. "Plasma Omega-3 Fatty Acids and Incident Diabetes in Older Adults." *Am J Clin Nutr* 94 (2): 527–33. doi:10.3945/ajcn.111.013334.

Forouhi, Nita G., Albert Koulman, Stephen J. Sharp, Fumiaki Imamura, Janine Kröger, Matthias B. Schulze, Francesca L. Crowe, et al. 2014. "Differences in the Prospective Association Between Individual Plasma Phospholipid Saturated Fatty Acids and Incident Type 2 Diabetes: The EPIC-InterAct Case-Cohort Study." *Lancet Diabetes Endocrinol* 2 (10): 810–18. doi:10.1016/S2213-8587(14)70146-9.

Giacca, Adria, Changting Xiao, Andrei I. Oprescu, Andre C. Carpentier, and Gary F. Lewis. 2011. "Lipid-Induced Pancreatic Beta-Cell Dysfunction: Focus on in Vivo Studies." *Am J Physiol Endocrinol Metab* 300 (2): E255–E262. doi:10.1152/ajpendo.00416.2010.

Kröger, Janine, Vera Zietemann, Cornelia Enzenbach, Cornelia Weikert, Eugène Hjm Jansen, Frank Döring, Hans-Georg Joost, Heiner Boeing, and Matthias B Schulze. 2011. "Erythrocyte Membrane Phospholipid Fatty Acids, Desaturase Activity, and Dietary Fatty Acids in Relation to Risk of Type 2 Diabetes in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study." *Am J Clin Nutr* 93 (1): 127–42. doi:10.3945/ajcn.110.005447.

Lankinen, Maria A., Alena Stančáková, Matti Uusitupa, Jyrki Ågren, Jussi Pihlajamäki, Johanna Kuusisto, Ursula Schwab, and Markku Laakso. 2015. "Plasma Fatty Acids as Predictors of Glycaemia and Type 2 Diabetes." *Diabetologia*, (Epub ahead of print). doi:10.1007/s00125-015-3730-5.

Lee, Joseph J., Jennifer E. Lambert, Yelena Hovhannisyan, Maria A. Ramos-Roman, Justin R. Trombold, David A. Wagner, and Elizabeth J. Parks. 2015. "Palmitoleic Acid Is Elevated in Fatty Liver Disease and Reflects Hepatic Lipogenesis." *Am J Clin Nutr* 101 (1): 34–43. doi:10.3945/ajcn.114.092262.

Ma, Wenjie, Jason H. Y. Wu, Qianyi Wang, Rozenn N. Lemaitre, Kenneth J. Mukamal, Luc Djoussé, Irena B. King, et al. 2015. "Prospective Association of Fatty Acids in the de Novo Lipogenesis Pathway with Risk of Type 2 Diabetes: The Cardiovascular Health Study." *Am J Clin Nutr* 101 (1): 153–63. doi:10.3945/ajcn.114.092601.

Rhee, Eugene P., Susan Cheng, Martin G. Larson, Geoffrey A. Walford, Gregory D. Lewis, Elizabeth McCabe, Elaine Yang, et al. 2011. "Lipid Profiling Identifies a Triacylglycerol Signature of Insulin Resistance and Improves Diabetes Prediction in Humans." *J Clin Invest* 121 (4): 1402–11. doi:10.1172/JCI44442.

Wang, Lu, Aaron R Folsom, Zhi-Jie Zheng, James S Pankow, John H Eckfeldt, and ARIC Study Investigators. 2003. "Plasma Fatty Acid Composition and Incidence of Diabetes in Middle-Aged Adults: The Atherosclerosis Risk in Communities (ARIC) Study." *Am J Clin Nutr* 78 (1): 91–98. `http://www.ajcn.org/cgi/pmidlookup?view=long&pmid=12816776`.

Wilke, M. S., M. A. French, Y. K. Goh, E. A. Ryan, P. J. Jones, and M. T. Clandinin. 2009. "Synthesis of Specific Fatty Acids Contributes to VLDL-Triacylglycerol Composition in Humans with and Without Type 2 Diabetes." *Diabetologia* 52 (8): 1628–37. doi:10.1007/s00125-009-1405-9.