Feasibility of Assessing Liver Lipid by Proton Magnetic Resonance Spectroscopy in Healthy Normal and Overweight Prepubertal Children

D. Enette Larson-Meyer, Ph.D., R.D., F.A.C.S.M.,¹ Bradley R. Newcomer, Ph.D. ARMTRIT,²
Connie L. VanVrancken-Tompkins, Ph.D.,³ and Melinda Sothorn, Ph.D.³

Abstract

Background: Intramyocellular and intrahepatic (IHL) lipids are significantly associated with insulin resistance in adults and adolescents and may represent an early marker for developing the metabolic syndrome or type 2 diabetes.

Methods: During the pilot phase of a larger cross-sectional study, we used proton magnetic resonance spectroscopy (¹H-MRS) to determine the feasibility of noninvasively evaluating IHL in 11 male ($n = 4$) and female ($n = 7$) prepubertal children using a standard clinical system and to determine whether IHL is correlated with adiposity, fasting insulin and glucose, and liver enzymes.

Results: Body mass index (BMI) (range, 13.4–32.4 kg/m²) and IHL stores (range, 0.07–3.2% relative to an oil phantom) were variable. IHL was correlated with body mass ($r = 0.66$, $P = 0.037$), BMI ($r = 0.73$, $P = 0.016$), percentage body fat ($r = 0.73$, $P = 0.01$, $n = 10$), waist circumference ($r = 0.85$, $P = 0.016$), and serum lactate dehydrogenase concentration ($r = 0.77$, $P = 0.03$) but was not significantly correlated with other markers of liver damage, including aspartate aminotransferase activity ($r = 0.59$, $P = 0.09$, $n = 9$) and alkaline phosphatase concentrations ($r = 0.60$, $P = 0.087$). IHL was also ($P < 0.01$) correlated with fasting insulin concentration ($r = 0.85$, $P = 0.03$, $n = 6$) and insulin resistance ($r = 0.94$, $P = 0.006$, $n = 6$), but these correlations were driven by the results for one child.

Conclusions: These preliminary data suggest that ¹H-MRS obtained in a standard pediatric clinical environment may be used to determine IHL in healthy normal and overweight prepubertal youth. This noninvasive technique may prove useful in identifying early markers of the metabolic syndrome in at-risk youth.

Introduction

LIPID ACCUMULATION within skeletal muscle (intramyocellular lipid [IMCL])^{1–5} and liver (intrahepatic lipid [IHL])^{6–9} (i.e., ectopic fat) is significantly associated with insulin resistance in adults and adolescents and may represent an early marker for developing the metabolic syndrome or type 2 diabetes mellitus (T2DM). Although it is not well established whether ectopic fat accumulates independent of, or as a consequence of, increased obesity, many studies have found correlations between increasing whole-body and/or abdominal adiposity and IMCL^{2,5,10} and IHL^{11,12} accumulation. IHL in addition is correlated with visceral abdominal adiposity in some^{7,11} but not all^{6,9} previous studies and

appears to be more sensitive to weight reduction via caloric restriction than is IMCL.⁷

Little is currently known, however, about ectopic fat accommodation and its relation to adiposity and insulin resistance before puberty. Using proton magnetic resonance spectroscopy (¹H-MRS), one study found that IMCL accumulated to a greater degree in prepubertal boys with a higher body mass index (BMI) and larger waist circumference and that such accumulation was related to the fasting glucose:insulin ratio.¹³ Further evaluation of ectopic fat, particularly IHL, is of timely importance given the increasing prevalence of insulin resistance^{14,15} and its potential long-term consequences in adulthood.¹⁶ Unfortunately, many of the techniques used to assess ectopic fat, including tissue biopsies

¹University of Wyoming, Laramie, Wyoming.

²Department of Clinical and Diagnostic Sciences, The University of Alabama at Birmingham, Birmingham Alabama.

³Louisiana State University Health Sciences Center, School of Public Health, New Orleans, Louisiana.

and computed tomography, are not appropriate for use in children because of their invasiveness or ionizing radiation exposure. ^1H -MRS, which is noninvasive and has been used successfully to assess IMCL, may be difficult to use in liver because it requires that participants lay motionless in a semi-enclosed tube during the procedure. Nevertheless, ^1H -MRS is a logical method for assessing IHL even in prepubertal children due to its safety (does not use ionizing radiation), availability at pediatric medical centers (providing purchase of the system's spectroscopy package), and proven reliability and validity.^{17,18} Thus, the primary purpose of this pilot study was to determine the feasibility of assessing IHL in healthy 7–9-year-old prepubertal children using a standard clinical MRS system. A secondary purpose was to determine whether IHL stores, if present, are correlated with adiposity, fasting insulin and glucose, and liver enzymes¹⁹ in such young healthy prepubertal children.

Subjects and Methods

Subjects

This pilot study was designed to examine the feasibility of conducting insulin sensitivity/resistance and ^1H -MRS testing in healthy prepubertal children with low birth weight, referred to as the SILLY study (Study of Insulin sensitivity in Louisiana Low-birth weight Youth). Subjects were the first children ($n = 11$) enrolled in the pilot study and were apparently healthy 7–9-year-old normal and overweight prepubertal children (Tanner stage <2) who were not taking regular prescribed medications. A screening blood sample confirmed their health status. The study was approved by the General Clinical Research Center, Children's Hospital, Louisiana State University Health Sciences Center, the Pennington Biomedical Research Center, and the University of Wyoming Institutional Review Boards, and written informed consent and assent were obtained from the child and his or her legal guardian.

Medical history, anthropometrics, blood sampling, and insulin resistance

Subjects were recruited from June 2005 through July 2006 and were tested at Children's Hospital in New Orleans, LA. Children were tested during a 3–4-h outpatient visit at Children's Hospital in New Orleans and a half-day visit to the General Clinical Research Center. During these visits, body mass and height were measured without shoes using standardized procedures. Waist circumference was measured at the level of the umbilicus. Insulin resistance was measured by the homeostatic model assessment (HOMA) methodology.²⁰ IHL was assessed by ^1H -MRS (as detailed below). Medical history was obtained from a family medical history questionnaire, which included information on the child's birth weight and current or past health problems. A fasting blood sample was drawn for analysis of liver function enzymes, insulin, and glucose.

^1H -MRS

Intrahepatic lipid was measured on a commercially available clinical 1.5-T whole-body imaging and spectroscopy system (Signa XL Horizon 2001 MRI Unit, General Electric, Medical Systems, Milwaukee, WI) located in the radiation

department of Children's Hospital, using the PRESS (Point RESolved Spectroscopy) technique. Children were positioned in a prone, feet-first orientation (i.e., on their belly, feet-first going into the magnet). An external amplitude reference

FIG. 1. (A) To assess IHL by ^1H -MRS, a single water-suppressed PRESS voxel ($3 \times 3 \times 3 \text{ cm}^3$) was positioned in an area of the liver's middle right lobe that was free from heavy vascularization as determined from T1-weighted axial scout images (25 slices, 8 mm thick, 2 mm separation). Although IHL content is determined by analysis of the water-suppressed spectra, individuals with higher IHL content can be visibly noted by viewing the non-water-suppressed spectra, which are shown for (B) the child with the lowest IHL content (0.07% relative to the oil phantom signal) (subject 08) and (C) the child with the highest IHL content (3.2%) (subject 02). Note that the image in (A) has been transposed for display purposes. Frequency is presented in parts per million.

TABLE 1. PHYSICAL CHARACTERISTICS, ANTHROPOMETRICS, PERTINENT LABORATORY VALUES, AND LIVER LIPID STORES IN PREPUBERTAL CHILDREN

Number	Sex	Age (years)	Mass (kg)	BMI		Body fat (%)	Waist (cm)	Birth mass (kg)	IHL (%)	AST (U/L)	ALKP (U/L)	LDH (U/L)	Insulin (μ U/mL)	Glucose (mmol/L)	HOMA ($\text{mM}/\mu\text{L}^2$)
				kg/m^2	z-score										
02	F	8	65.4	32.4	98	51.1		3.3	3.20	59	210	971	24.0	9.9	10.55
03	F	8	59.5	29.1	98	50.1		3.0	1.30						
05	F	8	24	15.5	39	15.8		2.8	1.50	30	188	560		4.3	
06	F	8	61.4	25.4	98	44.9	90.2	3.8	37	178	753		12.0	5.9	3.14
07	F	8	25	13.4	3	19.5	54.6	3.7	0.60	34	206		3.3	5.0	0.73
08	M	8	28	13.6	4	14.8	58.4	2.5	0.07	37	211	626	3.1	4.8	0.66
09	M	7	30.8	16.8	71	17.5	62.0	3.4	0.40	37	166	590	3.5	5.4	0.85
10	F	7	39.6	19.1	92	41.5	75.9	4.1	2.50	34	267	644		4.0	
11	M	9	36.2	15.4	28	24.3	62.2	3.3	0.10	35	150	536	11.0	4.4	2.17
13	F	8	29.6	17.0	66	25.0	61.0	2.9	0.90	39	204	605	3.0	4.4	0.59
14	M	8	31.8	15.9	48	26.4	61.5	2.7	0.60	35	193	593		4.5	
Mean		7.9	39.2	19.4	58.6	30.1	65.7	3.2	1.12	37.7	197.3	653.1	8.6	5.3	2.67
SD		0.5	15.4	6.5	36.7	14.0	11.6	0.5	1.04	7.9	31.7	134.2	7.9	1.7	3.61

Data are mean $\pm$ SD values. Insulin resistance was assessed by HOMA. M, male; F, female; AST, aspartate aminotransferase; ALK, alkaline phosphatase; LDH, lactate dehydrogenase.

phantom was created using a test tube filled with a known volume of peanut oil and was positioned vertically across the child's tailbone and secured with surgical tape. This placement helped assure that the phantom was placed close to the homogeneous spot of the magnet. A single water-suppressed PRESS voxel ($\sim 3 \times 3 \times 3 \text{ cm}^3$, echo time [TE] = 40 ms, repetition time [TR] = 1,500 ms) was then collected using the commercially provided ^1H -body coil using standard GE PRESS acquisitions in an area of the middle right lobe that was visually free from heavy vascularization as determined from T1-weighted axial images (25 slices, 8 mm thick, 2 mm separation; Fig. 1A) and not located too close to the surface subcutaneous fat. The larger voxel size allowed collection of less number of averages and a shorter scan time. Children were informed when the scan began and were asked to remain still and take shallow breaths during the procedure. An investigator practiced shallow breathing with the children before positioning in the magnet and also stressed the importance of remaining motionless during the acquisition. A single non-water-suppressed PRESS voxel was then collected from the phantom ($\sim 3 \times 1.5 \times 1.5 \text{ cm}^3$ voxel, TE = 40 ms, TR = 1,500 ms, 64 scans). To keep the children entertained during the 20-min procedure and to reduce the discomfort associated with the loud noises, children listened to personal music or story CDs via child-sized headphones.

Peak positions and areas of interest including the CH_2 (frequency = 1.4) and CH_3 (frequency = 1.0) fatty acid chain resonances were determined by time domain fitting using jMRUI (Java-Based Magnetic Resonance User Interface)¹⁹ by a single investigator. The residual water peak was removed using a HLSVD algorithm, and lipid resonances were fit using single lorentzian line shapes and referenced to the oil (phantom) resonance. The CH_2 resonance relative to the phantom (expressed as a percentage) was used as IHL.

Statistical procedures

Statistical analysis was performed using SPSS analysis software (SPSS version 13.0 for Windows, SPSS, Inc., Chicago,

IL). Data are presented as mean $\pm$ SD values. Pearson's correlation procedure was applied to determine associations between variables of interest. Significance was set at $\alpha > 0.05$.

Results

A usable liver spectrum was not available in one participant (patient 06) because of significant participant movement during data acquisition. This resulted in bad signal acquisition that produced a spectrum file with a low signal-to-noise ratio, which could not be analyzed. Data were also missing for several other parameters, including waist circumference and insulin resistance, because of staff shortages and blood transportation problems during post-Katrina recovery. Thus, the "n" values for all statistical analysis are reported in parentheses.

The characteristics of the 11 children, including their anthropometrics, blood measurements, and IHL content, are shown in Table 1. IHL stores were variable (range, 0.07–3.2%) and could also be detected visually in the non-water-suppressed spectra (Fig. 1). One participant (patient 02) was found to have elevated fasting glucose and insulin concentrations and was diagnosed with T2DM during the study. This participant was not dropped from the pilot analysis because our main purpose was to determine the feasibility of assessing IHL and determine if IHL accumulation occurs in prepubertal children. Correlations between IHL stores and fasting insulin and glucose and insulin resistance, however, were evaluated with and without this participant.

Liver lipid in relation to body size/composition, blood parameters, and insulin resistance

IHL was significantly correlated with body mass ($r = 0.66$, $P = 0.037$, $n = 10$), BMI (Fig. 2, upper panel; $r = 0.73$, $P = 0.016$, $n = 10$), percentage body fat (Fig. 2, middle panel; $r = 0.73$, $P = 0.01$, $n = 10$), waist circumference (Fig. 2, lower panel; $r = 0.85$, $P = 0.016$, $n = 7$), and serum lactate dehydrogenase concentration ($r = 0.77$, $P = 0.03$, $n = 8$) but was not significantly correlated with serum aspartate aminotransferase

FIG. 2. IHL was significantly correlated with (**upper panel**) BMI ($r = 0.73$, $P = 0.016$, $n = 10$), (**middle panel**) percentage body fat ($r = 0.73$, $P = 0.01$, $n = 10$), and (**lower panel**) waist circumference ($r = 0.85$, $P = 0.016$, $n = 7$).

activity ($r = 0.59$, $P = 0.09$, $n = 9$) and alkaline phosphatase concentration ($P = 0.60$, $P = 0.087$, $n = 9$). IHL was also ($P < 0.01$) correlated with fasting serum insulin concentration ($r = 0.85$, $P = 0.03$, $n = 6$) and insulin resistance ($r = 0.94$, $P = 0.006$, $n = 6$), but these correlations were driven by subject 02 and were not significantly correlated when this subject was removed from the analysis. IHL was not correlated with fasting glucose concentration ($r = 0.59$, $P = 0.09$, $n = 9$) and birth weight ($r = 0.46$, $P = 0.22$, $n = 9$).

Discussion

This pilot study demonstrates that noninvasive assessment of liver lipid by ^1H -MRS is feasible in healthy, normal and overweight prepubertal children and highlights the methodology of ^1H -MRS in a standard pediatric clinical environment. Most notably, our preliminary results are the first to demonstrate in exclusively prepubertal, healthy children who are both normal and overweight that many of the relations be-

tween IHL and components of the metabolic syndrome in adults and adolescents^{6-9,11,12} are also present in normal and overweight, apparently healthy prepubertal children. For instance, children with higher BMI and total adiposity and larger waist circumferences had increased IHL stores. Increased IHL stores, in turn, were significantly correlated with lactate dehydrogenase, a nonspecific marker of tissue damage (including liver).

Although the present pilot study demonstrated the feasibility of using ^1H -MRS to assess IHL in healthy normal and overweight youth, it is important to note, based on our pilot experience, that these measurements should be performed by a well-trained, dedicated clinical staff who are comfortable working with children. We originally anticipated losing some subject data due to movement artifacts (i.e., from wiggly participants). However, we were pleased that only one of 11 liver acquisitions was unusable because of motion artifact. Thus, 7-9-year-old children can successfully be kept "relatively" still and entertained during IHL acquisitions by stressing the importance of being still, practicing shallow breathing, and providing music/stories via headphones.

The most exciting findings from our pilot study were that IHL accumulation is initiated (at least in some children) before puberty and that this accumulation tends to be most noteworthy in those with presumed central adiposity and higher total adiposity. Although excessive liver fat accumulation, or hepatic steatosis, has been identified in ill or obese prepubertal^{21,22} and adolescent^{8,23} children, our studies are the first to identify this phenomenon noninvasively in healthy young prepubertal children. It is particularly interesting to note that IHL stores in the current study in children were mostly in the range we have previously seen in overweight, insulin-sensitive adults (range, 0.07-3.2% vs. 0.3-7.2%) using nearly identical methodology.⁷ Although the highest IHL accumulation was found in the child with newly diagnosed T2DM, it is clear that significant IHL stores were also present in other apparently healthy prepubertal children (Table 1). Certainly, these noninvasive measures should prove useful in identifying early markers of the metabolic syndrome in at-risk youth and also help to elucidate whether liver lipid accumulation is a cause or consequence of tissue insulin resistance. Ongoing research from this pilot is currently measuring both IHL and intramyocellular lipids by ^1H -MRS along with insulin sensitivity by the frequently sampled intravenous glucose tolerance test in a larger cohort of 400 healthy normal and overweight prepubertal African American and white children born with normal, high, and low birth weight.

In conclusion, while our study provides important preliminary results, they are limited by our small mixed sample of male and female white and African American children and our use of HOMA to assess insulin resistance. Regardless, these preliminary data suggest that ^1H -MRS may be successfully used to determine IHL in exclusively prepubertal, healthy, normal and overweight youth when assessed by a well-trained, dedicated staff who are comfortable working with children. Most importantly, IHL data may be successfully obtained in a standard pediatric clinical environment. This noninvasive measure may prove useful in identifying early markers of the metabolic syndrome in at-risk youth.

Acknowledgments

This work was supported by National Institute of Child Health and Human Development grant 1 R01 HD41071-01A2 entitled "Insulin Sensitivity in African-American and Caucasian Youth with Low Birth" and grant 1 R01 HD49046-01 entitled "Mechanisms of the Metabolic Syndrome in Pre-Pubertal Youth." Also, this study was supported in part by Tulane University and the Clinical and Translational Research Center, Louisiana State University Health Sciences Center as part of the Louisiana Board of Regents RC/EEP Fund (LSUHSC IRB 6134) and partially supported by CNRU Center grant 1P30 DK072476 entitled "Nutritional Programming: Environmental and Molecular Interactions" sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases.

Author Disclosure Statement

All authors declare that no competing financial interests exist.

References

- Pan D, Lillioja S, Kriketos A, Milner M, Baur L, Bogardus C, Jenkins A, Storlien L: Skeletal muscle triglyceride levels are inversely related to insulin action. *Diabetes* 1997;46:983-988.
- Forouhi NG, Jenkinson G, Thomas EL, Mullick S, Mierisova S, Bhonsle U, McKeigue PM, Bell JD: Relation of triglyceride stores in skeletal muscle cells to central obesity and insulin sensitivity in European and South Asian men. *Diabetologia* 1999;42:932-935.
- Jacob S, Machann J, Rett K, Brechtel K, Volk A, Renn W, Maerker E, Matthaei S, Schick F, Claussen CD, Haring HU: Association of increased intramyocellular lipid content with insulin resistance in lean nondiabetic offspring of type 2 diabetic subjects. *Diabetes* 1999;48:1113-1119.
- Perseghin G, Scifo P, De Cobelli F, Pagliato E, Battezzati A, Arcelloni C, Vanzulli A, Testolin G, Pozza G, Del Maschio A, Luzi L: Intramyocellular triglyceride content is a determinant of in vivo insulin resistance in humans: a ^1H - ^{13}C nuclear magnetic resonance spectroscopy assessment in offspring of type 2 diabetic parents. *Diabetes* 1999;48:1600-1606.
- Sinha R, Dufour S, Petersen KF, LeBon V, Enoksson S, Ma YZ, Savoye M, Rothman DL, Shulman GI, Caprio S: Assessment of skeletal muscle triglyceride content by ^1H nuclear magnetic resonance spectroscopy in lean and obese adolescents: relationships to insulin sensitivity, total body fat, and central adiposity. *Diabetes* 2002;51:1022-1027.
- Tiikkainen M, Tamminen M, Häkkinen AM, Bergholm R, Vehkavaara S, Halavaara J, Teramo K, Rissanen A, Yki-Järvinen H: Liver-fat accumulation and insulin resistance in obese women with previous gestational diabetes. *Obes Res* 2002;10:859-867.
- Larson-Meyer DE, Heilbronn LK, Redman LM, Newcomer BR, Frisard MI, Anton S, Smith SR, Alfonso A, Ravussin E: Effect of calorie restriction with or without exercise on insulin sensitivity, beta-cell function, fat cell size, and ectopic lipid in overweight subjects. *Diabetes Care* 2006;29:1337-1344.
- Perseghin G, Bonfanti R, Magni S, Lattuada G, De Cobelli F, Canu T, Esposito A, Scifo P, Ntali G, Costantino F, Bosio L, Ragogna F, Del Maschio A, Chiumello G, Luzi L: Insulin resistance and whole body energy homeostasis in obese adolescents with fatty liver disease. *Am J Physiol Endocrinol Metab* 2006;291:E697-E703.
- Seppälä-Lindroos A, Vehkavaara S, Häkkinen AM, Goto T, Westerbacka J, Sovijärvi A, Halavaara J, Yki-Järvinen H: Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. *J Clin Endocrinol Metab* 2002;87:3023-3028.
- Kautzky-Willer A, Krssak M, Winzer C, Pacini G, Tura A, Farhan S, Wagner O, Brabant G, Horn R, Stingl H, Schneider B, Waldhausl W, Roden M: Increased intramyocellular lipid concentration identifies impaired glucose metabolism in women with previous gestational diabetes. *Diabetes* 2003;52:244-251.
- Banerji MA, Buckley MC, Chaiken RL, Gordon D, Lebovitz HE, Kral JG: Liver fat, serum triglycerides and visceral adipose tissue in insulin-sensitive and insulin-resistant black men with NIDDM. *Int J Obes Relat Metab Disord* 1995;19:846-850.
- Luyckx FH, Lefebvre PJ, Scheen AJ: Non-alcoholic steatohepatitis: association with obesity and insulin resistance, and influence of weight loss. *Diabetes Metab* 2000;26:98-106.
- Ashley MA, Buckley AJ, Criss AL, Ward JA, Kemp A, Garnett S, Cowell CT, Baur LA, Thompson CH: Familial, anthropometric, and metabolic associations of intramyocellular lipid levels in prepubertal males. *Pediatr Res* 2002;51:81-86.
- Boney CM, Verma A, Tucker R, Vohr BR: Metabolic syndrome in childhood: association with birth weight, maternal obesity, and gestational diabetes mellitus. *Pediatrics* 2005;115:e290-e296.
- Kaufman FR: Type 2 diabetes mellitus in children and youth: a new epidemic. *J Pediatr Endocrinol Metab* May 2002;15(Suppl 2):737-744.
- Morrison JA, Friedman LA, Wang P, Glueck CJ: Metabolic syndrome in childhood predicts adult metabolic syndrome and type 2 diabetes mellitus 25 to 30 years later. *J Pediatr* 2008;152:201-206.
- Szczepaniak LS, Babcock EE, Schick F, Dobbins RL, Garg A, Burns DK, McGarry JD, Stein DT: Measurement of intracellular triglyceride stores by ^1H spectroscopy: validation in vivo. *Am J Physiol* 1999;276:E977-E989.
- Szczepaniak LS, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S, Hobbs HH, Dobbins RL: Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. *Am J Physiol Endocrinol Metab* 2005;288:E462-E468.
- Larson-Meyer DE, Newcomer BR, Heilbronn LK, Volaufova J, Smith SR, Alfonso AJ, Lefebvre M, Rood JC, Williamson DA, Ravussin E; Pennington CALERIE Team: Effect of 6-month calorie restriction and exercise on serum and liver lipids and markers of liver function. *Obesity (Silver Spring)* 2008;16:1355-1362.
- Cutfield WS, Jefferies CA, Jackson WE, Robinson EM, Hoffman PL: Evaluation of HOMA and QUICKI as measures of insulin sensitivity in prepubertal children. *Pediatr Diabetes* 2003;4:119-125.
- D'Adamo E, Impicciatore M, Capanna R, Loredana Marcovecchio M, Masuccio FG, Chiarelli F, Mohn AA: Liver steatosis in obese prepubertal children: a possible role

- of insulin resistance. *Obesity* (Silver Spring) 2008;16:677–683.
22. Schwimmer JB, Deutsch R, Kahen T, Lavine JE, Stanley C, Behling C: Prevalence of fatty liver in children and adolescents. *Pediatrics* 2006;118:1388–1393.
23. Cali AM, Zern TL, Taksali SE, de Oliveira AM, Dufour S, Otvos JD, Caprio S: Intrahepatic fat accumulation and alterations in lipoprotein composition in obese adolescents: a perfect proatherogenic state. *Diabetes Care* 2007;30:3093–3098.

Address correspondence to:
D. Enette Larson-Meyer, Ph.D., R.D., F.A.C.S.M.
Assistant Professor of Human Nutrition
Department 3354
University of Wyoming
1000 East University Avenue
Laramie, WY 82071

E-mail: enette@uwyo.edu