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E SAÚDE DA CRIANÇA

JOSÉ HERMES RIBAS DO NASCIMENTO

ACURÁCIA DA ULTRASSONOGRAFIA, UTILIZANDO A TÉCNICA COMPUTADORIZADA, NA AVALIAÇÃO DA DOENÇA HEPÁTICA GORDUROSA NÃO-ALCOÓLICA EM ADOLESCENTES OBESOS E EUTRÓFICOS, COMPARATIVAMENTE COM A RMN E CORRELAÇÃO COM EXAMES LABORATORIAIS

Porto Alegre
2012

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Tese de Doutorado apresentado ao curso de Pós-Graduação em Medicina/Pediatria e Saúde da Criança da Pontifícia Universidade Católica do Rio Grande do Sul, como parte dos requisitos necessários à obtenção do título de Doutor em Medicina/Pediatria.

Orientador:

Prof. Dr. Matteo Baldisserotto

Porto Alegre

2012

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Porto Alegre

2012

Dedicatória

Para Nathália e Gabriela, minhas filhas, que me ensinam a cada dia a simplicidade da vida e do amor.

AGRADECIMENTOS

Durante a realização deste trabalho pude contar com o auxílio de várias pessoas que se tornaram referências como colaboradores e amigos e que, além da contribuição técnica e científica, confirmaram sua importância na minha vida.

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Aos meus pais João e Elaine por terem me ensinado a sonhar e a participar da vida com suas perdas e ganhos.

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Aos pacientes e suas famílias que diariamente nos estimulam a aprimorar a arte do cuidar e nos trazem ensinamentos constantes.

RESUMO

Objetivos: Avaliar a acurácia da ultrassonografia, utilizando a análise computadorizada na DHGNA, em adolescentes obesos e eutróficos: correlação com a RMN e exames laboratoriais.

Métodos: O estudo transversal prospectivo avaliou cinquenta adolescentes (11 a 17 anos), sendo 24 obesos e 26 eutróficos. Todos os adolescentes foram submetidos aos exames de US com a análise computadorizada, RMN, exames laboratoriais e avaliação antropométrica.

Foi calculada a sensibilidade, a especificidade, os valores preditivos positivo e negativo e a acurácia, seguindo a posterior geração de curva ROC e cálculo da área sob a curva estabelecida, para determinar o melhor ponto de corte para o Gradiente Hepatorrenal Ultrassonográfico(GHRU), a fim de predizer graus de esteatose, utilizando os resultados da RMN como padrão-ouro.

As medidas da insulina, fibrinogênio, fosfatase alcalina (FA), triglicerídeos, Gama GT, colesterol total e frações HDL e LDL, aminotransferases, proteína C-reativa (PCR) e cálculo do HOMA-IR *(homeostatic model assessment of insulin resistance índex*) foram analisados e distribuídos com uma longa curva caudal à direita (+). Foram utilizados testes não-paramétricos nas análises relacionadas com essas variáveis.

Resultados: O grupo dos obesos, era composto por 29,2% de meninas e 70,8% de meninos e, o grupo de eutróficos, por 69,2% de meninas e 30,8% de meninos. A média da idade dos obesos e eutróficos foi de 14,2 anos (±2;11-17 anos) e 14,7 anos(±2;12-17anos), respectivamente. Observou-se 19,2% de pacientes eutróficos com esteatose e 83% de obesos com esteatose. A prevalência de esteatose nos obesos foi significativamente maior (83,3%; IC 95%: 64,5% - 94,5%) do que nos eutróficos (19,2%; IC 95%: 7,4% a 37,6%).

Após a análise do GHRU, que foi calculado pela diferença de ecogenicidade entre o parênquima do fígado e a ecogenicidade do córtex renal, verificou-se diferença significativa entre a mediana dos adolescentes obesos e eutróficos (mediana= 19,5; P25=15,5; P75=28 vs mediana: 10,0; P25=8; P75=11; $p<0,001$). Foi observada, ainda, diferença significativa entre a mediana dos adolescentes com e sem esteatose (mediana=22; P25=18,5; P75=29,5 vs mediana=9; P25=7,8; P75=10; $p<0,001$).

A curca ROC gerada para o GHRU, foi com ponto de corte de 13 com uma sensibilidade de 100% e uma especificidade de 100% . Considerando esse mesmo ponto de corte para os eutróficos, obter-se-íam 9,5% de falsos positivos (especificidade=90,5%) e 0% de falsos negativos (sensibilidade=100%).

Os adolescentes obesos apresentaram valores laboratoriais mais elevados do que os eutróficos, exceto para o colesterol HDL (p<0,001). Os adolescentes com DHGNA apresentaram valores laboratoriais mais elevados do que os adolescentes sem DHGNA. Na análise multivariada , apenas o HOMA-IR aparece como um fator de risco independente de DHGNA.

Conclusões: Sendo o US com a análise computadorizada e o cálculo do GHRU uma técnica simples para avaliação quantitativa da ecogenicidade hepática e não invasiva, o presente estudo sugere que esse procedimento pode tornar-se importante no seguimento de adolescentes obesos e eutróficos com DHGNA. Esse método poderá, ainda, servir no rastreamento populacional da DHGNA e em estudos clínicos.

Palavras-chave: Adolescente, Obesidade, Doença Hepática Gordurosa Não-alcoólica, Gradiente Hepatorrenal, Ultrassonografia, Ressonância Magnética nuclear, HOMA.

ABSTRACT

Purpose: Evaluate the accuracy of ultrasonography by using computerized analysis in the NAFLD (NonAlcoholic Fatty Liver Disease) in obese and eutrophic adolescents: correlation with MR and laboratorial exams.

Method: The prospective cross-sectional study evaluated fifty adolescents (from 11 to 17 years old) being 24 obese and 26 eutrophic ones. The adolescents were submitted to US exams with computerized analysis, MR, laboratorial exams and anthropometric evaluation.

Sensibility, specificity, positive and negative predictive values and accuracy were calculated following ROC curve and area calculation under the established curve to determine the best Ultrasonographic Hepatorenal Gradient (UHRG) to predict steatosis using MR results as gold standard.

Insulin, fibrinogen, alkalyne phosphatase (AF), triglycerides, GT Gama, aminotransferases, C-reactive protein (CRP) and HOMA-IR (*homeostatic model assessment of insulin resistance índex*) measures were analyzed and distributed through a long right (+) caudal curve. Non parametric tests were used in the analysis related to these variables.

Results: In the obese group 29.2% were girls and 70.8% were boys and in the eutrophic group 69.2% were girls and 30.8% were boys. The obese and eutrophic age range was 14.2 years old (±2;11-17 years old) and 14,7 (±2;12-17anos), respectively. The eutrophic patients with steatosis represented 19,2% and the obese ones represented 83% with steatosis. The prevalence of steatosis in the obese patients was significantly higher (83.3%; IC 95% : 64.5%) than in the eutrophic ones (19.2%; IC 95% : 7.4% - 37.6%).

After the analysis of the UHRG, calculated through the difference in ecogenicity between liver cortical and the liver parenchyma echogenicity a significant difference was revealed among the mean obese and eutrophic adolescents (mean = 19.5; P25=15.5; P75=28 vs mean: 10.0; P25=8; P75=11; p<0,001). A significant difference was also observed between adolescent with and without steatosis (median=22; P25=18.5; P75=29.5 vs median=9; P25=7,8; P75=10; p<0,001).

The ROC curve generated for the UHRV estimated a cutoff point 13 and sensibility and specificity estimated in 100%. Considering the same cutoff point for

the eutrophic, 9.5% false positives would be estimated (specificity =90.5%) and 0% false negatives (sensibility = 100%).

The obese adolescents presented higher laboratorial values when compared with the eutrophic ones with the exception of cholesterol HDL ($p<0,001$). The adolescents with NAFLD presented higher lab values than the adolescents without NAFLD. In the multi varied analysis only the HOMA-IR poses a risk factor regardless of NAFLD.

Conclusions: Being the ultrasonography with computerized analysis and hepatorenal gradient a simple quantitative noninvasive technique for hepato echogenicity, it can be of great support in the follow-up of obese and eutrophic adolescents with NAFLD. Such method can be useful to trace population with NAFLD and also for clinical studies.

Keywords: Adolescent, Obesity, NonAlcoholic Fatty Liver Disease, Hepatorenal Gradient, Ultrasonography, Nuclear Magnetic Resonance, HOMA-IR.

LISTA DE FIGURAS

CAPÍTULO I

CAPÍTULO II

CAPÍTULO III

LISTA DE TABELAS

CAPÍTULO II

CAPÍTULO III

LISTA DE ABREVIATURAS

CA	Circunferência Abdominal
CT	Colesterol Total
DHGNA	Doença Hepática Gordurosa Não Alcoólica.
EHNA	Esteato Hepatite Não Alcoólica
FA	Fosfatase Alcalina
FG	Fração de Gordura
G-GT	Gama glutamil transferase
GHRU	Gradiente Hepatorrenal Ultrassonográfico
HDL	High-density lipoprotein
HOMA-IR	Homeostatic Model Assessment - Insulin Resistence
IMC	Indice de Massa Corporal
OMS	Organização Mundial da Saúde
PCR	Proteína C-reativa
RMN	Ressonância Magnética Nuclear
ROC	Receiver Operating Characteristic
TC	Tomografia Computadorizada
TGO	Transaminase Glutâmica Oxalacética
US	Ultrassom

SUMÁRIO

CAPÍTULO I

APRESENTAÇÃO

JUSTIFICATIVA

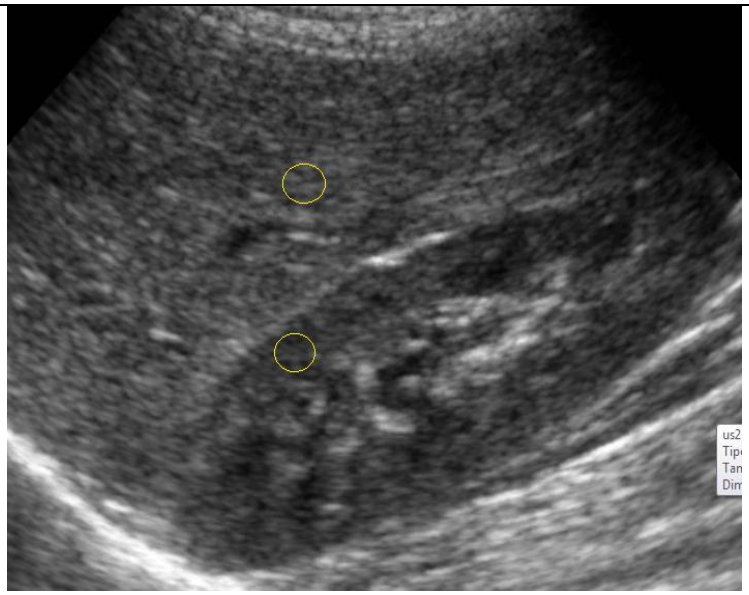
OBJETIVOS

REFERÊNCIAS

1.1 APRESENTAÇÃO

Em razão do grande aumento na incidência e na prevalência de obesidade na população adolescente, existe a necessidade de ser realizado o diagnóstico precocemente de Doença Hepática Gordurosa Não-alcoólica (DHGNA) por meio de métodos não invasivos(1). Na presente tese, é investigada uma nova técnica para a avaliação ultrassonográfica da DHGNA. Ela consiste, no cálculo do GHRU, em um método para avaliação objetiva da DHGNA, utilizando um software de análise computadorizada de domínio público *Image J* (rsd.info.nih.gov/ij/index.html) que mede a ecogenicidade da imagem digital obtida por meio da ultrassonografia. Esse software quantifica a ecogenicidade do parênquima hepático e do córtex renal obtidos pelo ultrassom sendo seus valores graduados em escada de cinza, variando de 0 até 255, do preto ao branco, respectivamente, método este já descrito por Soder et al.(2). Após a mensuração da ecogenicidade nas regiões de interesse, os dois valores resultantes são subtraídos para obtenção do gradiente hepatorrenal ultrassonográfico(GHRU) .Fig.1 e Fig.2.

PACIENTE SEM ESTEATOSE



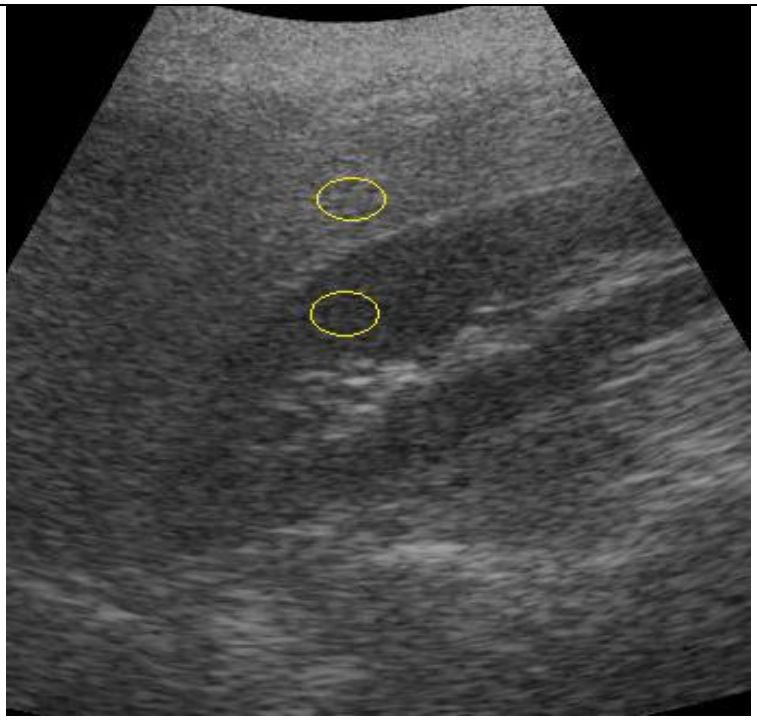
Área fígado: 400 pixels, média: 43.3

Área Rim: 300 pixels; média:34,8

Cálculo do GHRU: 8,5

Figura 1. Imagem A. Imagens ultrassonográficas demonstrando as regiões de interesse. Ultrassonografia do fígado em paciente adolescente eutrófico. Gradiente hepatorrenal (*sem esteatose).*

PACIENTE COM ESTEATOSE



Área fígado: 400pixels, média: 76,8

Área Rim: 300 pixels; média:43,0

Cálculo do GHRU: 33,8

Figura 1. Imagem B -Imagens ultrassonográficas demonstrando as regiões de interesse. Ultrassonografia do fígado em paciente adolescente obeso. Gradiente hepatorrenal (com esteatose).

Importante lembrar que o uso do US com técnica de análise computadorizada, como método para a avaliação da DHGNA, é a forma mais acessível para rastreamento populacional(2).

No presente trabalho avaliou-se a prevalência de DHGNA em uma população de adolescentes obesos e eutróficos, assim como a correlação com o perfil

antropométrico e exames laboratoriais.

A tese compõe-se dos seguintes capítulos: introdução (apresentação, justificativa e objetivos), desenvolvimento (artigos original 1 e original 2) e conclusão. Os artigos recebem os seguintes títulos:

Artigo original 1 - acurácia da ultrassonografia, utilizando a técnica computadorizada, na avaliação da doença hepática gordurosa não-alcoólica, em adolescentes obesos e eutróficos, comparativamente com a RMN.

Artigo original 2 - adolescentes obesos e eutróficos com doença hepática gordurosa não-alcoólica, diagnosticados pela RMN, apresentam aumento do homa-ir.

1.2 JUSTIFICATIVA

A prevalência do sobrepeso e da obesidade vem drasticamente aumentando em todo o mundo, em especial em crianças e adolescentes (3), (4), provavelmente justificando o grande aumento da DHGNA como a principal causa de doença hepática na população pediátrica ao redor do mundo(5,6). Há um crescimento epidêmico da DHGNA com estimativas de 80 milhões a 100 milhões de norte-americanos afetados(7). No Brasil, a real prevalência da DHGNA ainda é desconhecida estimando-se uma prevalência de 18% na população geral(8), representando, nas últimas décadas, um grande problema de saúde pública, sendo considerada uma epidemia global pela Organização Mundial da Saúde (OMS)(9). Nos países europeus a DHGNA tem crescido em torno de 10 a 40% nos últimos 10 anos(10, 11). A crescente epidemia de obesidade na adolescência tem orientado pediatras na investigação das condições relacionadas a essa doença (12).

Recentemente, Soder R, et al. descreveram uma nova técnica para o diagnóstico da DHGNA, utilizando o US com análise computadorizada, quantitativa, com o cálculo do GHRU que apresentou ótimos resultados em crianças obesas e eutróficas(2), porém essa técnica não foi comparada com nenhum outro método de imagem.

Salienta-se que, até o momento, não foram realizados estudos clínicos comparando a acurácia do GHRU, no diagnóstico da DHGNA, com a RMN, que é o padrão-ouro em análise de imagem.

Em virtude do US com análise computadorizada ser um método reprodutível, acessível e de baixo custo para avaliação da DHGNA, o presente estudo visa comparar a acurácia do US com análise computadorizada mediante cálculo do GHRU, com a quantificação obtida pela RMN, na investigação da DHGNA em adolescentes obesos e eutróficos, objetivando a validação da primeira técnica.

Além da busca por um método não invasivo que apresente uma alta acurácia e precisão no diagnóstico da DHGNA em pacientes pediátricos e adolescentes, é de fundamental importância a utilização de uma técnica que apresente baixo custo e nenhuma radiação em virtude dos potenciais efeitos nocivos da radiação ionizante(8).

1.3 OBJETIVOS

Principal:

1. Avaliar a acurácia da ultrassonografia, utilizando a análise computadorizada, na DHGNA em adolescentes obesos e eutróficos: correlação com a RMN.

Secundários:

1. Quantificar a DHGNA em adolescentes obesos e eutróficos por meio da RMN e da US, mediante a análise computadorizada e cálculo do GHRU.
2. Avaliar a prevalência da DHGNA em adolescentes obesos e eutróficos.
3. Avaliar as propriedades diagnósticas do método ultrassonográfico computadorizado comparado à RMN (padrão-ouro), estabelecendo valores de sensibilidade e de especificidade para diferentes pontos de corte.
4. Avaliar a associação da DHGNA com perfil antropométrico e laboratorial.

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CAPÍTULO II

2.1 ARTIGO ORIGINAL I

ACURÁCIA DA ULTRASSONOGRAFIA, UTILIZANDO A TÉCNICA COMPUTADORIZADA, NA AVALIAÇÃO DA DOENÇA HEPÁTICA GORDUROSA NÃO- ALCOÓLICA, EM ADOLESCENTES OBESOS E EUTRÓFICOS, COMPARATIVAMENTE COM A RMN

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American Journal of Roentgenology

ACCURACY OF ULTRASOUND USING A COMPUTERIZED TECHNIQUE TO EVALUATE NONALCOHOLIC FATTY LIVER DISEASE IN OBESE AND EUTROPHIC ADOLESCENTS AS COMPARED TO NMRI

--Manuscript Draft--

Manuscript Number:	
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Article Type:	Original Research
Section/Category:	Pediatric Imaging
Keywords:	Ultrasound, Nuclear Magnetic Resonance, obesity,nonalcoholic fatty liver disease, adolescents
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Abstract:	Objective: To compare the accuracy of ultrasound (US) with computerized analysis of the hepatorenal gradient (HRG) to nuclear magnetic resonance imaging (NMRI) for the evaluation of NAFLD in adolescents Material and methods: This prospective transversal study evaluated 50 adolescents (aged 11-17 years), including 24 obese and 26 eutrophic individuals. All adolescents underwent computer-based US, NMRI, laboratory exams, and anthropometric evaluation. Posterior generation of the receiver operating characteristic (ROC) curve was performed, and the area under the ROC curve was calculated to determine the cutoff point for the HRG and to predict levels of steatosis. The sensitivity, specificity, positive and negative predictive values, and accuracy of US with computerized analysis were compared with those of NMRI as the gold standard. Results: The obese group included 29.2% girls and 70.8% boys, and the eutrophic group included 69.2% girls and 30.8% boys. The prevalence of NAFLD was 19.2% for the eutrophic group and 83% for the obese group. The ROC generated for the HRG, with a cutoff point of 13, had 100% sensitivity and 100% specificity. Use of this same cutoff point for the eutrophic group led to a false positive rate of 9.5% (90.5% specificity) and false negative rate of 0% (100% sensitivity). Conclusion: US with computerized analysis of the HRG is a simple and noninvasive technique for the quantitative evaluation of hepatic ecogenicity that could be used to help identify obese and eutrophic adolescents with NAFLD. This method could be used in population tracking for NAFLD and for clinical studies.
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	Marylin J Siegel, MD, PhD Professor, Pediatric Radiology, washingont university of school medice, St. Louis MO siegelM@mir.wustl.edu
Opposed Reviewers:	

Cover Letter

12/09/2012

Dear Professor

Please find attached our original manuscript entitled "ACCURACY OF ULTRASOUND USING A COMPUTERIZED TECHNIQUE TO EVALUATE NONALCOHOLIC FATTY LIVER DISEASE IN OBESE AND EUTROPHIC ADOLESCENTS AS COMPARED TO NMRI" that we would like to submit to the Editorial Board of the *American Journal Radiology*. This manuscript has not been published or is under active consideration by another journal. All authors have approved this submission and there are no conflicts of interest involved.

Because of the large increase in the incidence and prevalence of obesity among adolescents, early diagnosis of nonalcoholic fatty liver disease (NAFLD), using non-invasive methods is necessary. Therefore, we compare the accuracy of ultrasound (US), computed with the analysis and calculation of the gradient hepatorenal (HRG) with magnetic resonance imaging (NMRI) in the assessment of NAFLD in adolescents. This method could be used following the population for NAFLD and clinical studies, with low cost for its diagnosis.This justify the importance of this work.

Yours Sincerely,

José Hermes Ribas do Nascimento, MD

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Full Title Page

ACCURACY OF ULTRASOUND USING A COMPUTERIZED TECHNIQUE TO EVALUATE NONALCOHOLIC FATTY LIVER DISEASE IN OBESE AND EUTROPHIC ADOLESCENTS AS COMPARED TO NMRI

Various factors have stimulated the development and prioritization of research concerning noninvasive methods to diagnose nonalcoholic fatty liver disease (NAFLD) (1). In recent decades, an increase in the prevalence of excess weight and obesity has led to a great increase in NAFLD as the main cause of liver disease within the pediatric population worldwide (2, 3). There is a rising NAFLD epidemic, with estimates of 80 to 100 million Americans affected (4). In Europe, cases have increased from around 10% to 40% in the last 10 years (5). Studies about childhood obesity, which use clinical, biochemical, and ultrasonic parameters to grade fatty infiltration of the liver, have found rates of prevalence for hepatic steatosis between 53% and 77% (6, 7). The prevalence and severity of NAFLD are tied to ethnic factors, with increased levels for the population of Mexican-American children and in people of Hispanic origin than for other ethnic groups (8).

Hepatic steatosis is a broad term used to denote the accumulation of lipids, especially triglycerides, in the cytoplasm of hepatocytes, to levels exceeding 5% of the weight of the liver (9, 10). NAFLD covers a wide range of changes, in which steatosis is the fundamental element of the disease. Steatosis may progress to fibrosis in up to 41% of cases, according to Ekstedt et al. (11-13). There are currently no specific biochemical markers or blood tests to diagnose NAFLD. The most accurate diagnostic method of NAFLD is liver biopsy, which provides important information about the degree of liver compromise, global changes to the liver

architecture, and severity of the inflammation process and fibrosis (14). However, because of its invasive nature, liver biopsy cannot be used to track a population on a large scale, nor can it even be used to track patients after treatment because of the risks associated with the procedure (15).

To date, various imaging techniques have been used to detect hepatic steatosis, including ultrasound (US), computerized tomography (CT), magnetic resonance imaging (MRI), and spectroscopic nuclear magnetic resonance imaging (SNMRI) (15, 16). Although CT has good accuracy and allows a semiquantitative diagnosis, its applicability to monitor response to treatment is limited because it involves the use of ionizing radiation (17). Spectroscopic MRI is currently the most accurate technique available, detecting a quantity of fat lower than 0.5%. However, it has some limitations, such as a long procedural time, high cost, need for an experienced operator, and requirement of data processing and interpretation of the results. Therefore, SNMRI is of limited use in clinical practice for population studies and for monitoring treatment. Although NMRI is highly accurate and sensitive and, therefore, seems more appropriate for general use, it is still an expensive technique and the exam is lengthy (17, 18).

Ultrasound is a low-cost option, although it uses a subjective analysis to evaluate steatosis and, thus, does not provide quantitative data. As a result, the sensitivity of US is reduced to about 60%, especially for obese patients or those who have little fatty infiltration of the liver (7, 19). Soder et al. recently described a new technique to diagnose NAFLD, using US with a computerized, semiquantitative analysis to calculate the hepatorenal gradient (HRG). This method yielded excellent results for obese and eutrophic children (20). However, they did not compare this technique with any other imaging method.

To date, no clinical study has been performed to compare the accuracy of the HRG calculated by computerized US to diagnose NAFLD with NMRI as the gold standard in terms of

image analysis. Ultrasound with computerized analysis has been shown to be a reproducible, accessible, and inexpensive method for diagnosing NAFLD. Therefore, the aim of this study was to compare the accuracy of US with computerized analysis in calculating the HRG with quantification by NMRI for the investigation of NAFLD in obese and eutrophic adolescents, to validate the use of US with computerized analysis.

MATERIALS AND METHODS

Study population

A prospective transversal study involving 50 adolescents (aged 11–17 years) was performed between October 2011 and February 2012. The study participants included 24 obese adolescents, who were seen at the walk-in Nutritional Clinic of the Regional University of Ijuí (Universidade Regional de Ijuí, UNIJUÍ), and 26 eutrophic adolescent students, who were volunteers from a public school in a town in Região das Missões, Rio Grande do Sul State. The adolescents underwent NMRI exams, laboratory exams, and anthropometric evaluation to diagnose steatosis. Adolescents with hepatorenal disease, those using hepatotoxic drugs, nephrotoxins, corticosteroids, or immunosuppressants, and those with other chronic diseases were excluded from the study.

A nutritionist with 10 years of experience and with the rank of specialist examined the selected patients who came to nutrition clinic at UNIJUÍ. The medical coordinator of the multidisciplinary team performed the laboratory exams, which were obtained up to 1 week after the anthropometric measurements, US, and NMRI were performed. Laboratory exams were performed at the cooperative medical laboratory of the state of Rio Grande do Sul (UNIMED/RS).

A medical radiologist with extensive experience in the area of abdominal x-rays and with a rank equivalent to that of an American specialist performed the US exams with computerized analysis at the DIAGEM medical clinic in the city of Ijuí, using a General Electric (GE) machine (Milwaukee model, manufactured in 2009). On the same day, the NMRI exams were performed on a GE machine (Signa Excite, manufactured in 2009) with a 1.5-T magnet, 4-channel phase array surface coils, and linear array coil.

The ethics committee of the Pontifical Catholic University of Rio Grande do Sul (Pontifícia Universidade Católica do Rio Grande do Sul, PUCRS) approved the study. Written informed consent was obtained from all of the parents or legal guardians of the adolescents.

Anthropometric measurements

For each adolescent, the sex, age, weight, height, and abdominal circumference were recorded, and the body mass index (BMI) was calculated as the weight (kg) divided by height (m^2). The body mass index (BMI) table of the National Center for Health Statistics (NCHS) versus age was used to calculate the BMI for age percentile for each adolescent. A BMI $\geq 97^{th}$ percentile was used for the group with obesity, and a BMI between the 25^{th} and 75^{th} percentiles (P25 and P75, respectively) was used for the eutrophic group. To compare BMIs across ages and sexes, the Z score was used, which represents the number of standard deviations (SDs) above and below the average values of the population for children and adolescents. Obesity was diagnosed as a z-score > +2, and eutrophia was diagnosed as a z-score > -2 and < +1.

Liver US technique with computerized analysis

The US exams were performed in real time with gray scale by using a convex 3.5–5 MHz transductor on a GE platform, with adjusted image parameters for all of the adolescents examined. For each patient, two representative images of the liver parenchyma and the right renal cortex were obtained by using a subcostal approach on the midclavicular line. The US images included the VI liver segment and the adjacent renal parenchyma, obtained at the level of the hepatorenal space during maximum inhaling.

The US images, which were initially acquired in DICOM format, were digitized. Computerized analysis was performed with Image J software, which is available in the public domain (rsd.info.nih.gov/ij/index.html). From the digital image, the ecogenicity values of the liver parenchyma and the right renal cortex were measured, with the values graded on a gray scale ranging from 0 (black) to 255 (white). Two circular regions of interest (ROIs) were selected: the liver parenchyma, near its lower edge (area: 400–500 pixels), and the right renal cortex, near its upper edge (area: 300–400 pixels). The ROIs were selected, as previously described by Soder et al. (20), in a juxtaposed way and on the same axis of the acoustic beam, so as to minimize artifacts and variations in the ecogenicity of the acoustic window. The two resulting ecogenicity values of the ROIs were subtracted to obtain the ultrasonic hepatorenal gradient (UHRG). This protocol was applied to all adolescents included in the study, whether obese or eutrophic (Figure 1).

The images were recorded in DICOM format and analyzed by two medical radiologists to check the agreement of the values.

Figure 1 –Image A and B

Image A

Imagem B

Hepatic NMRI technique

For all participants, liver NMR images were obtained with a 1.5-tessel NMRI machine on a GE platform. Images were acquired on the axial plane during the end of exhalation, in apnea, to reduce the overall acquisition time to approximately 15 seconds. To calculate the fat fraction (FF), the Dixon two-point method as modified by Fishbein et al. (18) was used, as previously described by Pacifico et al. (21). The FF was calculated from the difference in signal intensity (SI) between the two vectors resulting from the in-phase (IP) and out-of-phase (OP) signals.

The parameters for the multiple sequences of acquisitions in T1, echo gradient, were as follows: repetition time, 174 milliseconds; echo time, 2.1 milliseconds for OP and 4.9 milliseconds for IP images; field of vision, 35 cm × 40 cm; cortical thickness, 5 mm; flip angle, 70°; matrix size, 256 × 182. For calculating the SI values for the liver and spleen, ROIs were acquired in IP and OP images, with a circular average for the ROIs of 1 cm^2 (21) (Figure 2).

Liver fat was quantified as the percentage relative to the loss of the SI of the liver in OP images, by using the following formula: FF = [SI IP – SI OP / 2 × SI IP] × 100, where SI is the average SI of the liver divided by the average SI of the spleen. The SI of the spleen was used as a denominator in the formula to adjust for the lack of an objective SI scale for NMRI, a method previously used by Pacífico et al. (21-23) (Table1).

The ROIs were drawn on segment VI of the liver, near the cortical area, taking care to avoid areas with vessels, motion artifacts, and the partial volume effect. Similar actions were performed for the spleen, and the average SI was calculated for the ROIs of the liver.

Figura 2 (A and B)

Image A

Image B

Table 1-

Statistical analysis

Recorded data, which were stored in Excel spreadsheets, were analyzed by statistical methods with the SPSS package, version 18.0 (Statistical Package for the Social Sciences). A *p*-value < 0.05 was considered to be statistically significant. Continuous data are reported as the mean ± SD (in the case of symmetric distribution) or median and interquartile range (in the case of asymmetric distribution). Categorical data are presented as absolute frequencies and proportions. Differences between averages were evaluated with Student's *t*-test (in the case of normal distribution) or the Mann-Whitney test (in the case of abnormal distribution). For the evaluation of categorical variables, the Chi-square test was used. To evaluate interobserver agreement, the Friedman test with the intraclass correlation coefficient (ICC) was used. To evaluate intraobserver agreement, the Wilcoxon test with the ICC was used.

To evaluate the diagnostic properties of the UHRG, posterior generation of the receiver operating characteristic (ROC) curve was performed. The area under the curve was established to determine the cutoff point for the UHRG and to predict grades of steatosis. The sensitivity,

specificity, positive and negative predictive values, and accuracy were calculated by using the NMRI results as the gold standard. The selected cutoff points prioritized the minimization of false positive and negative values.

Sample size calculation

To calculate the sample size, data from previous studies were used, which evaluated the accuracy and quantified the fat in children via NMRI (8, 20, 24). On the basis of these previous studies, 50 individuals were needed to obtain the estimated FF by NMRI and the gradient inferred by US for a linear correlation coefficient (Pearson's r) of the order of 0.5, significance level of 5%, and statistical power of 90% (4, 20).

Results

The obese group included 7 girls (29.2%) and 17 boys (70.8%). The eutrophic group included 18 girls (69.2%) and 8 boys (30.8%). The obese group had a higher proportion of boys than the eutrophic group ($p = 0.011$). Average ages for the obese and eutrophic groups were 14.2 ± 2 years (11–17 years) and 14.7 ± 2 years (12–17 years), respectively. The obese group showed a higher percentage of adolescents with increased abdominal circumference compared to the eutrophic group (95.8% vs. 15.4%; $p < 0.001$). The prevalence of steatosis in the obese group was higher (20 patients, 83.3%; CI 95%: 64.5% to 94.5%) than that in the eutrophic group (5 patients, 19.2%; CI 95%: 7.4% to 37.6%).

The UHRG was calculated based on the difference between the ecogenicity values of the liver cortical area and the renal parenchymal area. The median UHRG differed between obese

and eutrophic adolescents (median = 19.5, P25 = 15.5, P75 = 28 vs. median = 10.0, P25 = 8, P75 = 11; $p < 0.001$). However, when adolescents with and without steatosis were divided into groups and the difference between obese and eutrophic patients was re-evaluated, there was no significant difference between them (with steatosis: $p = 0.818$; without steatosis: $p = 0.971$; Fig. 3). We observed a significant difference between the median for adolescents with and without steatosis (median = 22, P25 = 18.5, P75 = 29.5 vs. median = 9, P25 = 7.8, P75 = 10; $p < 0.001$)(Figura 3).

Figura 3-

The ROC curve generated for the UHRG is shown in Fig. 4, with a cutoff point of 13, 100% sensitivity, and 100% specificity (Figure 4). Using this same cutoff point, we obtained a false positive rate of 9.5% (90.5% specificity) and false negative rate of 0% (100% sensitivity). A computerized analysis of the digital images did not show any statistically significant differences for the hepatic parenchymal ecogenicity values between the obese and eutrophic groups (87.6 ± 25.5 vs. 82.5 ± 32, $p = 0.535$) or for the renal ecogenicity values between the groups (67.3 ± 31.2 vs. 73.9 ± 32.5, $p = 0.466$) (Table 2).

Figure 4-

Table 2-

Validation of the US technique with computerized analysis

The US exams performed on 20 patients who participated in this research were randomly separated and re-evaluated by 3 medical radiologists with more than 10 years of experience in abdominal radiology to calculate the UHRG. The results showed no significant difference among the three evaluators (Friedman test; $p = 0.100$) and revealed a high ICC (0.95; CI 95%: 0.90 to

0.98). There was no difference between two evaluations by the same observer (Wilcoxon test; $p = 0.275$), with a high ICC (0.97; CI 95%: 0.93 to 0.99).

DISCUSSION

In this study, the prevalence of NAFLD was 19.2% for the eutrophic group and 83% for the obese group. These findings are similar to those of Chan et al., who found a prevalence of hepatic steatosis between 53% and 77% (7). However, a study by Pacifico et al. (2005) on 55 obese children in Italy found a prevalence for steatosis of 40%, as diagnosed by NMRI (24). According to Devadason et al. (2012), who performed a broad review of NAFLD and NASH(Nonalcoholic steatohepatitis), there are limited studies about the prevalence of these pathologies in children and adolescents, due to the practical difficulty of diagnosing them. Both disorders require invasive biopsies and exams, like NMRI, that are very expensive and not performed in routine clinical practice (25).

A study by Soder et al. evaluated a new method to quantify NAFLD by US with computerized analysis. Through this method, the ecogenicity of the liver and renal parenchyma can be measured to calculate the gradient. That study, which analyzed obese and eutrophic children, showed significant differences in the median UHRG between the two groups analyzed, although this diagnostic method was not compared with any other imaging method (20). In the present study, we also obtained significant differences for the median UHRG values between obese and eutrophic adolescents (19.3 vs. 10.0; $p < 0.001$), similar to the results obtained by Soder et al. (33.9 ± 6.6 vs. 14.1 ± 6).

We observed a significant difference between the median UHRG values for adolescents with and without steatosis (22 vs. 9; $p < 0.001$). However, when we divided the adolescents into groups with and without steatosis and re-evaluated the difference between obese and eutrophic patients, we did not identify any significant difference between them (with steatosis: $p = 0.818$; without steatosis: $p = 0.971$). Calculation of the ROC curve generated by the UHRG defined a cutoff point of 13, with 100% sensitivity and 100% specificity in predicting steatosis. Use of this same cutoff point for the eutrophic subjects led to false positive and false negative rates of 9.5% and 0%, respectively (90.5% specificity). The accuracy of the method was 100%. Studies performed by Saadeh et al. using conventional, subjective US demonstrated its high sensitivity in detecting severe steatosis (>33%), but with a positive predictive value of 62% and a weak interobserver evaluation for mild and moderate steatosis (26). Strauss et al. showed low interobserver ($K = 0.43$) and intraobserver agreement ($k = 0.54$) with conventional US (27).

Fishbein et al. (2005) observed an excellent correlation in the quantification of liver fat via liver biopsy (puncture for steatosis) and NMRI in adults with predominantly macrovesicular steatosis associated with NAFLD. In Pacifico et al. (2007), both NMRI and US correlated well with the microscopic fat content, and serious steatosis was evident on US in all cases; however, US was unable to delineate the liver fat content. In particular, US was limited in its ability to show the gradations of steatosis compared to quantitative evaluation of the FF by NMRI (24). In that previous study, children with moderate to severe steatosis by US showed a wide range of liver fat contents within both categories by NMRI. These findings suggested that the usefulness of US was limited because of its inability to identify fat regression or progression in individuals with NAFLD. Thus, if a child with NAFLD had a reduction in FF by NMRI from 40% to 20%

because of a very successful intervention, US would probably not show a corresponding change in appearance (24).

In this study, because of the small number of patients with NAFLD diagnosed by NMRI (25 adolescents), it was not possible to grade the NAFLD as mild, moderate, or severe. In future studies with larger samples of patients, we should be able to use the UHRG calculation to grade NAFLD based on US, as we have done with NMRI, via the FF calculation.

In the present study, a single measurement of the ecogenicity of the liver ($p = 0.534$) and of the renal cortex ($p = 0.466$) did not show significant differences between obese and eutrophic patients. However, when we calculated the UHRG (i.e., the difference between the ecogenicity of the liver and the renal cortex), we detected a significant difference between the obese and eutrophic groups (19.3 vs. 10.0; $p < 0.001$). This finding may be explained by the fact that there was little or no effect of obesity on the renal cortex. Unlike the liver, which is infiltrated by fat, the renal cortex is a constant parameter for calculation of the UHRG.

One limitation of the study was the small number of obese and eutrophic patients in the analysis. Because of this small number, overweight patients were not included in this study. Another clear limitation is the question of whether we were studying an altered renal parenchyma associated with a pre-existing parenchymatous renal disease, which could change the US evaluation and calculation of the UHRG compared to the liver. Similarly, the presence of liver fibrosis in some patients could make the linear correlation between fatty infiltration and renal ecogenicity unreliable.

We observed that UHRG was highly accurate in diagnosing NAFLD for the groups of adolescents studied. This finding suggested that this method may reduce the high interobserver and intraobserver variations associated with traditional US. Studies with the same objective,

which evaluated the importance of measuring liver and renal ecogenicity values, were conducted by Jeong et al. on 54 patients with mild, moderate, and severe steatosis and on patients without steatosis. They used a gray-scale histogram, with complex parameters and calculations that would be difficult to apply in everyday clinical practice, but obtained good results in terms of sensitivity and specificity when comparing the scores for steatosis (28). Because US with computerized analysis (such as with Image J and UHRG calculation) is a simple and noninvasive technique for the quantitative analysis of liver ecogenicity, it could become a way to track obese and eutrophic adolescents with NAFLD. The method offers a shorter examination time, without exposure to ionizing radiation as in CT, and with reduced costs for public health systems. This method could also be used to track the population with NAFLD and for clinical studies.

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Table 1. Diagnostic accuracy of NMRI to diagnosis mild, moderate, and severe steatosis, as described by Pacifico et al. (21)

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Hepatic steatosis	**≥ 5%** **Grade I, mild**	**≥ 33%** **Grade II, moderate**	**≥ 66%** **Grade III, severe**
Cutoff point	4.85	9	19
Area under the curve	0.98 (95% CI, 0.98–1.0)	1	1
Sensitivity (%)	95.8	100	100
Specificity (%)	100	100	100

Table 2. Accuracy of ultrasound with computerized analysis compared to NMRI as the gold standard

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Hepatic steatosis	**Total sample**	**Eutrophic**	**Obese**
Cutoff point	13.0	14.5	13.0
Area under the curve	1.00 (1.00 – 1.00)	1.00 (1.00 – 1.00)	1.00 (1.00 – 1.00)
Sensitivity (%)	100	100	100
Specificity (%)	100	100	100
Accuracy (%)	100	100	100

Figure 1. Liver ultrasound images of patients without steatosis (A) and with steatosis (B) showing regions of interest (yellow circles) for hepatorenal gradient (UHRG) calculation.

Figure File (.tiff, .jpg)

Figure 1. Liver ultrasound images of patients without steatosis (**A**) and with steatosis (**B**) showing regions of interest (yellow circles) for hepatorenal gradient (UHRG) calculation. A, Eutrophic adolescent patient without steatosis. Liver area: 400 pixels (average: 43.3 pixels). Rim area: 300 pixels (average: 34.8 pixels). UHRG: 8.5. B, Obese adolescent patient with steatosis. Liver area: 400 pixels (average: 76.8 pixels). Rim area: 300 pixels (average: 43.0 pixels). UHRG: 33.8.

Figure A

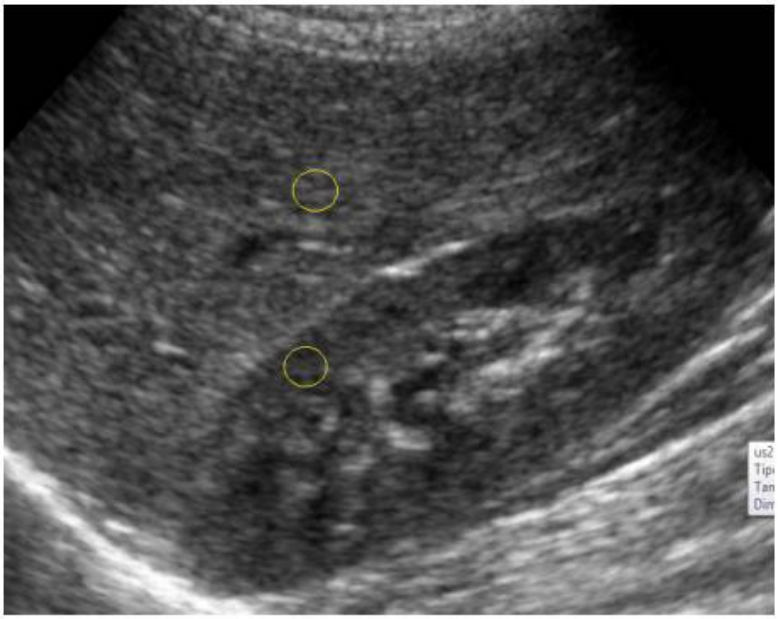

Figure 1. Liver ultrasound images of patients with steatosis (B) showing regions of interest (yellow circles) for hepatorenal gradient (UHRG) calculation.

Figure B

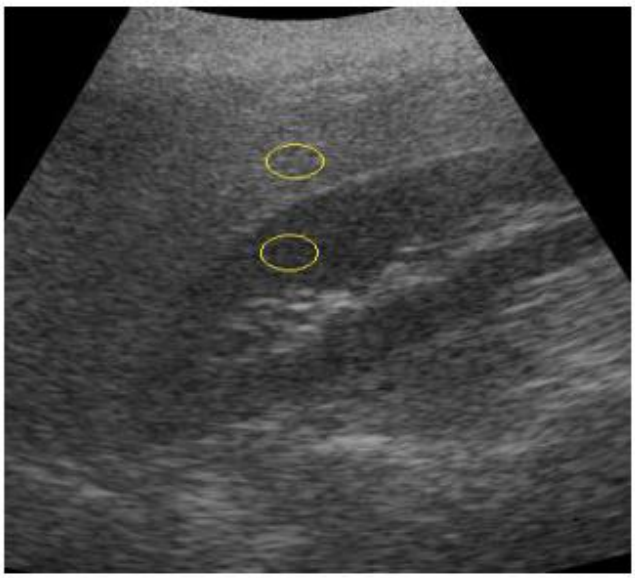

Figure 2. NMRI images of a patient with NAFLD, demonstrating the in-phase and out-of-phase signal intensity, the chemical shift, and the ROIs.

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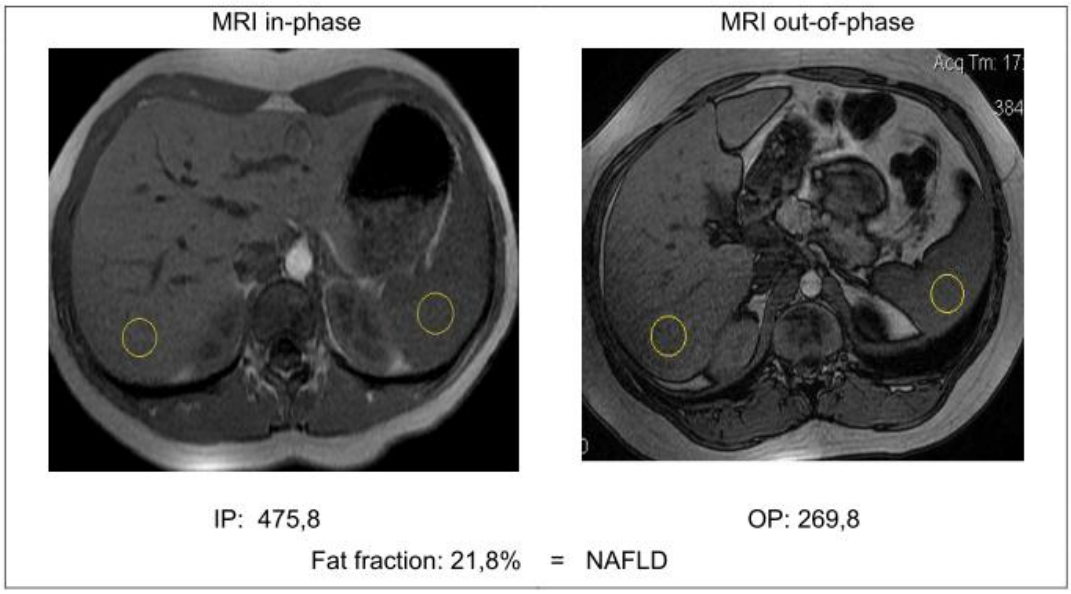

Figure 2. NMRI images of a patient without NAFLD, demonstrating the in-phase and out-of-phase signal intensity, the chemical shift, and the ROIs.

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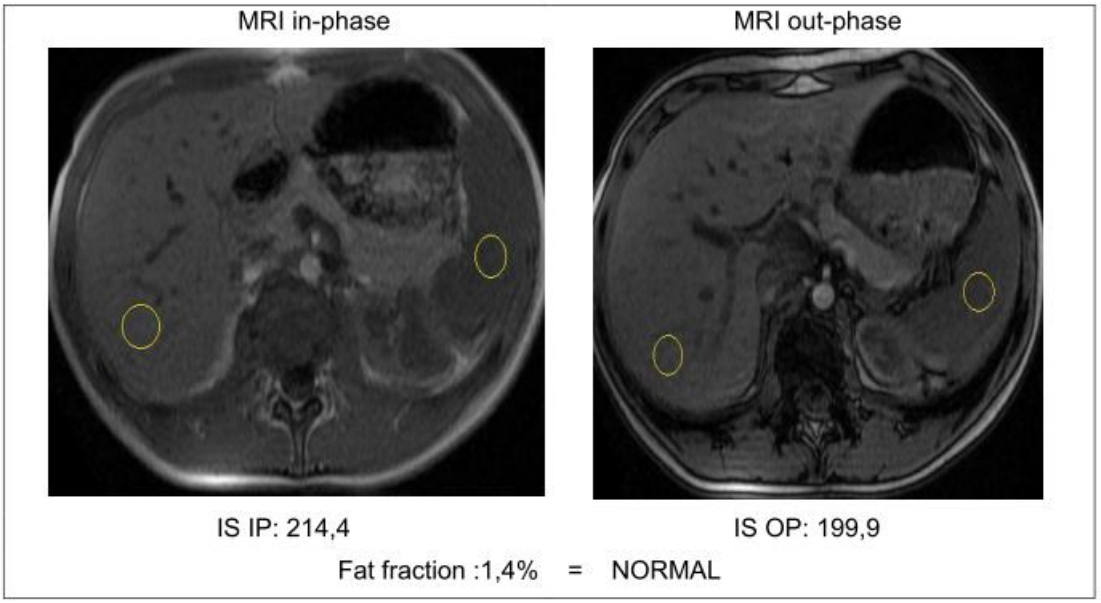


Figure 3. Box plot of the values (levels) of the hepatorenal gradients (HRGs) between the obese and eutrophic groups in individuals with and without steatosis. The line within the box represents the median. Upper and lower limits of the box represent the 75th and 25th percentiles (P75 and P25), respectively. Error bars above and below represent the median ± 1.5 (P75–P25).

Figure 3. Box plot of the values (levels) of the hepatorenal gradients (HRGs) between the obese and eutrophic groups in individuals with and without steatosis.

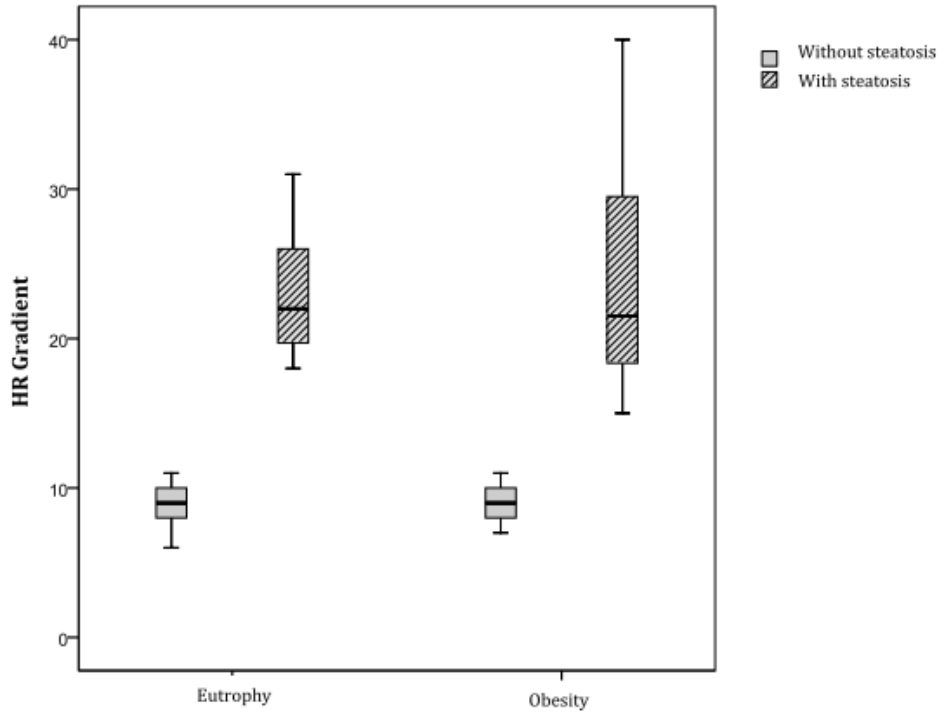
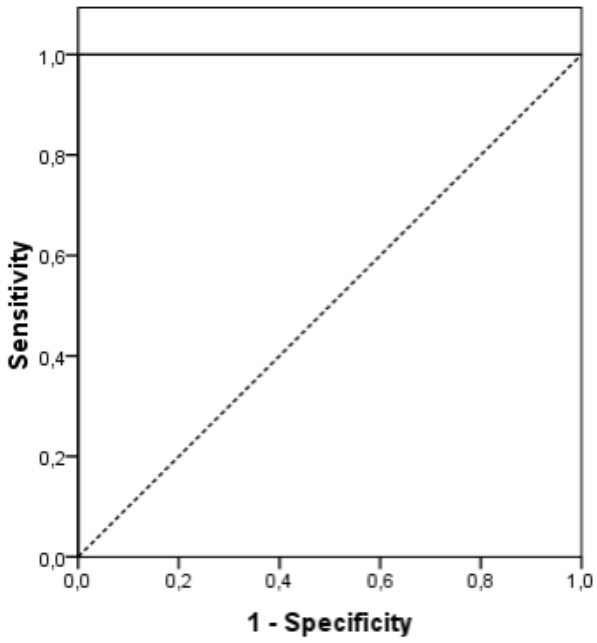


Figure 4. Receiver operating characteristic (ROC) curve, for evaluating the cutoff point of the UHRG, considering NMRI as the gold standard for steatosis.

Figure 4. Receiver operating characteristic (ROC) curve, for evaluating the cutoff point of the UHRG, considering NMRI as the gold standard for steatosis.



CAPÍTULO III

ARTIGO ORIGINAL II

3.1 ARTIGO ORIGINAL II

ADOLESCENTES OBESOS E EUTRÓFICOS COM DOENÇA HEPÁTICA GORDUROSA NÃO ALCOÓLICA, DIAGNOSTICADOS PELA RMN, APRESENTAM AUMENTO DO HOMA.

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Keywords: Nuclear magnetic resonance imaging (NMRI),non-alcoholic fatty liver disease (NAFLD),adolescents, insulin resistance (HOMA), obesity.

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Abstract: OBJECTIVES: To evaluate the presence of non-alcoholic fatty liver disease (NAFLD) in obese and eutrophic (non-obese) adolescents with nuclear magnetic resonance imaging (NMRI) and its relationship to insulin resistance and other potential biomarkers.

METHODS: A total of 50 adolescents (aged 11-17 years), including 24 obese adolescents and 26 eutrophic adolescents were evaluated using NMRI exams for NAFLD diagnosis. Blood analysis was performed to measure glucose, insulin, total cholesterol, HDL cholesterol, triglycerides, fibrinogen, aminotransferases, alkaline phosphatase, gamma-gt, and C-reactive protein. The HOMA-IR (homeostatic model assessment of insulin resistance) index was also calculated. Laboratory test results and anthropometric assessment, were statistically analyzed to determine potential correlation with NAFLD prevalence.

RESULTS: Within the obese group, 29.2% were girls and 70.8% boys, and in the eutrophic group, 69.2% were girls and 30.8% boys. The average age for the obese and eutrophic groups was 14.2 (±2; 11-17 years old) and 14.7 (±2; 12-17 years old), respectively. The prevalence of NAFLD among the obese was significantly higher (83.3%; CI 95%: 64.5-94.5%) than that of the eutrophic group (19.2%; CI 95%: 7.4-37.6%). Compared to eutrophic adolescents, the obese adolescents had significantly higher levels for all parameters measured except for total and HDL cholesterol, which were significantly lower. In multivariate analysis, only HOMA-IR was an independent risk factor for NAFLD.

CONCLUSION: The prevalence of NAFLD was 19.2% among eutrophic patients and 83.3% among obese patients. Only HOMA-IR was determined to be an independent risk factor for NAFLD.

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Opposed Reviewers: We have not oppose reviewers We have not oppose reviewers
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A. Cover Letter

11.16.2012

Dear Professor

Please find attached our original manuscript entitled **"Insulin resistance is a predictive factor for MRI-diagnosed non-alcoholic fatty liver disease in obese and eutrophic adolescents"** that we would like to submit to the Editorial Board of the *Journal of adolescent health* . This manuscript has not been published or is under active consideration by another journal. All authors have approved this submission and there are no conflicts of interest involved.

We evaluate the presence of non-alcoholic fatty liver disease (NAFLD) in obese and eutrophic (non-obese) adolescents with nuclear magnetic resonance imaging (NMRI) and its relationship to insulin resistance and other potential biomarkers . Because of the high prevalence of obesity and non-alcoholic fatty liver disease in this age group and the need for more accurate diagnostic methods and low cost for its diagnosis, justify the importance of this work.

Yours Sincerely,

José Hermes Ribas do Nascimento, MD

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Insulin resistance is a predictive factor for MRI-diagnosed non-alcoholic fatty liver disease in obese and eutrophic adolescents

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD), also called hepatic steatosis, was first described in adult patients in 1980 by Ludwig et al. as a clinicopathologic condition which presents histology findings that correspond with alcoholic hepatitis, but in people who do not drink (1). Cases of NAFLD have risen drastically in the United States, with estimates of 80 to 100 million Americans currently being affected (2, 3). In Europe, growth in the percentage of the population afflicted with NAFLD has grown by 10–40% in the last 10 years (4). Recent studies have shown an association between NAFLD and childhood obesity (5, 6), with estimated prevalence rates being lower than 10% for the general population, but 70~75% for the obese juvenile population (7, 8). Geographically, NAFLD prevalence is particularly high in the United States (3, 9). The disease is more common in males, with the average age of diagnosis being between 11 and 13 years of age. The higher prevalence among boys this age tends to be explained by the fact that it coincides with a period of greater resistance to insulin, which is common during puberty (10, 11).

In adults, insulin resistance is recognized as a central factor in the development of type-2 diabetes and has been associated with obesity, metabolic syndrome, hypertension, and heart disease (12, 13). It is also clear that insulin resistance contributes significantly to obesity and cardiometabolic risk in children and adolescents. However, there is currently no consensus on strategies for prevention or treatment of insulin resistance (12, 13).

Because of the high prevalence of NAFLD in the adolescent and pre-adolescent population in both developed and developing countries, it is believed that this disease is one of the most common causes of chronic hepatopathia for these age groups (14). Today, there is evidence that NAFLD is associated with a significant increase in global mortality rate as well as in the incidence of cardiovascular disease independent of classic atherosclerotic risk factors. These observations suggest that NAFLD is not just a marker, but is also an early mediator in atherosclerosis (15). Thus, early detection and intervention in cases of NAFLD in childhood and adolescence is critical.

Nuclear magnetic resonance imaging (NMRI) provides highly accurate NAFLD diagnosis. However, to date, there have been few studies using NMRI to evaluate the liver of obese adolescents or eutrophic (non-obese) adolescents. The majority of NAFLD studies to date used ultrasound imaging, which involves a subjective analysis susceptible to high inter- and intra-observer variation (16, 17). Therefore, the goal of this study was to evaluate a group of obese adolescents and a group of eutrophic adolescents using NMRI to diagnose NAFLD and compare results with blood analysis data to determine potential biomarkers of NAFLD.

Materials and Methods

Study population

This study was performed between October 2011 and February 2012, with 50 adolescents (aged 11 to 17). The group included 24 obese adolescents, seen at the walk-in Nutritional Clinic of the Regional University of Ijuí (Universidade Regional de Ijuí, (UNIJUÍ) and a second group of 26

eutrophic adolescent students, volunteers from a public school in a town in Região das Missões, Rio Grande do Sul State. The adolescents underwent NMRI exams, laboratory tests, and anthropometric evaluation in order to diagnose NAFLD and quantify potentially correlated biomarker levels.

The ethics committee of the Pontifical Catholic University of Rio Grande do Sul (Pontifícia Universidade Católica do Rio Grande do Sul, PUCRS) approved the study, and we obtained informed written consent from all the adolescents' parents or legal guardians.

Anthropometric measurements

A nutritionist with 10 years of experience and the rank of specialist examined the selected patients at UNIJUÍ's nutrition clinic. Data collected included sex, age, weight, height and abdominal circumference. The body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). We used the NCHS's (National Center for Health Statistics) BMI vs. age table to calculate each adolescent's BMI percentile.

To compare BMIs across ages and sexes, we calculated the Z score, the number of standard deviations above and below the population's average values. We categorized patients with a BMI equal to or greater than the 97^{th} percentile (z-score > 2) as obese and patients with a BMI between the 25^{th} and 75^{th} percentile (-2 > z-score < 1) in the eutrophic group.

Laboratory tests

The medical coordinator for our multidisciplinary team requested the laboratory exams, which were performed up to a week after the anthropometric

measurements and the NMRI. The cooperative medical laboratory of the state of Rio Grande do Sul (UNIMED/RS) performed all the laboratory exams.

Each study participant fasted for 12 hours before the laboratory exams, for more accurate measurement of the concentrations of glucose, insulin, total cholesterol, HDL cholesterol, triglycerides, fibrinogen, aminotransferases, alkaline phosphatase (AP), gamma-gt and C-reactive protein. Blood samples were collected and analyzed according to standardized procedures.

The HOMA-IR (homeostatic model assessment of insulin resistance) index was calculated to determine resistance to insulin and the functional capacity of the pancreatic beta cells. The HOMA-IR, which is based on the glucose and insulin fasting levels, as described in 1985 by Matthews et al., was calculated as follows: (fasting insulin- µU/mL) x (fasting glucose–mmol/L)/22.5. Insulin resistance was defined as HOMA IR > 2 (13, 18, 19).

Nuclear magnetic resonance imaging of the liver.

A medical radiologist with more than 10 years of experience in the area of abdominal x-rays, with a rank equivalent to that of an American specialist, performed the MRIs at the DIMAGEM medical clinic in the city of Ijuí. The radiologist used a 1.5-T Signa Excite, manufactured in 2009, with 4-channel phase array surface coils and a linear array coil (GE Healthcare). Images were acquired on the axial plane at the end of exhalation, in apnea, to reduce the overall acquisition time to approximately 15 s.

The parameters for the multiple sequences of acquisitions in T1 echo gradient imaging mode were as follows: repetition time of 174 ms, echo time 2.1 ms for out-of-phase (OP) images and 4.9 ms for in-phase (IP) images; field of

vision, 35 cm x 40 cm; cortical thickness, 5 mm; 70° flip angle; matrix size, 256 x 182. Regions of interest (ROIs) with average size of 1 cm^2 were defined for both liver and spleen in OP and IP images (20). We drew the ROIs on segment VI of the liver, near the cortex, taking care to avoid areas with vessels, motion artifacts, and partial volumes. Similar measures were performed for the spleen, and the average SI was calculated for both organs. The liver SI was normalized to the spleen's SI to account for the lack of an objective SI scale for NMRI (20-22).

To calculate the fat fraction (FF), we used the two-point Dixon method, as modified by Fishbein et al. (23) and previously described by Pacifico et al. (20). Liver fat was quantified as the percentage loss of liver signal intensity (SI) in OP images relative to IP images, using the following formula: $FF = [SI_{IP} - SI_{OP} / 2 \times SI_{IP}] \times 100$, where SI is the average of the liver's signal intensity divided by the spleen's average signal intensity, and SI_{IP} and SI_{OP} are the in-phase and out-of-phase signal intensity, respectively.

Figure 1-

Figure 2-

Table 1-

Exclusion criteria

We excluded from the study any participants with hepatorenal disease or other chronic diseases. We also excluded participants who used hepatotoxic drugs, nephrotoxic drugs, corticosteroids, or immunosuppressant agents.

Statistical analysis

The data was recorded, inspected in graph and table form, and then analyzed using SPSS (Statistical Package for the Social Sciences, version 18.0, IBM). A p-value < 0.05 was considered statistically significant.

Measurements of insulin, fibrinogen, alkaline phosphatase, triglycerides, gamma-gt, GOT and HOMA IR were analyzed and distributed along a long-run supply curve to the right (+). Therefore, we used non-parametric tests for the analyses of these variables. Differences between the averages were evaluated using Student's t-test for variables with normal distribution or, in the case of abnormal distribution, the Mann-Whitney test. We applied the Chi-square test to evaluate the categorical variables.

To avoid confusion factors and to evaluate variables independently associated with NAFLD, the multivariate Poisson regression model was applied. To measure the effect of each measured variable, prevalence ratios with 95% confidence interval were calculated. To minimize the effects of multicolinearity (an association between independent variables), two models were generated: one with obesity and the other with abdominal circumference.

Results

The patients were divided into obese and eutrophic (healthy weight) study groups according to anthropometric measurements (Table 2). Study participants were also divided, based on NMRI–measured liver FF, into patients with NAFLD and patients without NAFLD (Table 3). The obese group contained 7 girls (29.2%) and 17 boys (70.8%) while the eutrophic group included 18 girls (69.2%) and 8 boys (30.8%). The average age for the obese and the eutrophic

groups was 14.2 years old (±2; 11–17 years) and 14.7 years old (±2; 12–17 years), respectively. The obese group had a significantly higher proportion of boys than the eutrophic group (p = 0.011). The percentage of patients with high abdominal circumference was lower for the eutrophic group than for the obese group (15.4% vs. 95.8%, respectively; p < 0.001).

In our study, the prevalence of NAFLD among the obese was significantly higher (83.3%; 95% CI: 64.5–94.5%) than among the eutrophic subjects (19.2%; 95% CI: 7.4–37.6%). Compared to eutrophic adolescents, obese adolescents had significantly higher levels of glucose, HOMA-IR, AP, fibrinogen, GOT, Gamma GT and CRP, but significantly lower levels of total and HDL cholesterol. There was no significant difference between groups for total cholesterol or triglycerides (Table 2).

Table 2-

We observed that compared to adolescents without NAFLD, adolescents with NAFLD had significantly higher values for BMI, AC, glucose HOMA-IR, AP, GOT, fibrinogen, but lower levels of HDL cholesterol. There was no significant difference between the NAFLD and non-NAFLD groups for total cholesterol, Gamma GT, CRP, or triglycerides (Table 3).

Table 3-

To account for the multicolinearity effect, we performed two Poisson regression models, one for nutritional state and the other for abdominal circumference. After adjusting for multivariate models (Table 4), only HOMA > 2 presented itself as an independent risk factor for NAFLD (Figure 3).

Table 4-

Figure 3-

Discussion

Because of the current obesity epidemic, studies on NAFLD and insulin resistance in children and adolescents have become an important source of debate and research for professionals in this area of health. This prospective transverse study evaluated 50 adolescents (24 obese and 26 eutrophic) using NMRI to diagnose NAFLD and compared results with standard blood analysis results in an attempt to find correlated biomarkers for future use in diagnosis.

We found that the prevalence for NAFLD among the children in our obese study group (20/24; 83.3%) was significantly higher than that in the eutrophic group (5/26; 19.2%). The measured prevalence for the obese group in our study was slightly higher than values described by Franzese et al. and Chan et al., who found prevalence for NAFLD among obese adolescents to be 53% and 77%, using clinical, biochemical, and ultrasound parameters to grade the fatty infiltration of the liver (7, 8). We know that the prevalence and severity of NAFLD is associated with ethnic factors, with there being a higher prevalence in the population of Mexican-American children and in people of Hispanic origin than in other ethnic groups, and that body fat distribution and insulin sensitivity can also vary by race and ethnicity (24).

We observed that adolescents with NAFLD had significantly higher values than adolescents without NAFLD in the categories of BMI, AC, glucemia HOMA-IR, AP, GOT, and fibrinogen. Recent articles have observed a close association between NAFLD and abdominal obesity, atherogenic dyslipidemia, hypertension, insulin resistance and glucose intolerance, all of which are associated with metabolic syndrome. These studies report that 90% of patients

with NAFLD have at least one characteristic of metabolic syndrome and 33% have a complete diagnosis, with NAFLD being the liver's manifestation of metabolic syndrome (25). Various studies have confirmed a relationship between NAFLD and the characteristics of metabolic syndrome in adults (26, 27). Evidence for the relationship between metabolic syndrome and NAFLD in children is also emerging (28).

A controlled case study comparing 150 overweight children with NAFLD diagnosed by biopsy with 150 children without NAFLD discovered that after adjusting for age, sex, race, ethnicity and hyperinsulinemia, children with metabolic syndrome had an OR (Odds rations) of 5.0 (95% CI: 2,6-9,7) for NAFLD compared to the children without metabolic syndrome(29) . This is the most convincing data to date to support a significant relationship between NAFLD and metabolic syndrome, which cannot be inferred solely by the coexistence of obesity in these two conditions. These findings support the hypothesis that an accumulation of fat in the liver plays an important role in the pathogenesis of obesity and other co-morbidities (30).

Using multivariate analysis, in this study, we identified only HOMA-IR>2 as an independent risk factor for NAFLD. However, there seems to be an interaction between obesity and altered HOMA-IR. For high HOMA-IR, the prevalence of NAFLD is similar between the obese group (86.4%) and eutrophic group (83.3%). However, if HOMA-IR is low, the prevalence of NAFLD is higher in the obese group (50%) than in the eutrophic group (0%), with a borderline descriptive sample level (p = 0.091). Kumashiro et al. suggested that NAFLD associated with an increase in insulin resistance is caused by increased liver diacylglycerol (DAG) in lipid droplets observed in anatomopathology and that

NAFLD is a better predictor of insulin resistance in humans(31). The authors also noted that the association between insulin resistance and NAFLD makes NAFLD an important factor in the pathogenesis of type 2 diabetes. The development of liver insulin resistance has been attributed to various causes, including inflammation, endoplasmic reticulum (ER) stress, and the accumulation of hepatocellular lipids in animal models of NAFLD. Kelly et al. observed an independent association between NAFLD and insulin resistance, demonstrating that the presence of NAFLD influences the severity of type-2 diabetes. In type-2 diabetes, insulin sensitivity is markedly reduced and is correlated with the degree of liver fat infiltration measured through computed tomography and visceral fat infiltration measured through magnetic resonance (32).

Adults suspected to have NAFLD, diagnosed on the basis of elevated blood liver enzymes and US imaging, show an increase in insulin resistance, even when they have a normal body weight. According to these data, insulin resistance is a reproducible marker for the development of NAFLD in obese as well as eutrophic adults (25).

Schwimmer et al. reported that an increase in insulin resistance was nearly universal in a study group, composed mostly obese American adolescents, with biopsy-proven NAFLD (33). In that study, even after individuals with diabetes were excluded, nearly all children with NAFLD exhibited insulin resistance. Mandato et al. reported that insulin resistance was approximately four times more common in 20 obese children with an increase in aminotransferases and NAFLD, diagnosed by US imaging, in comparison to 30 age-matched control subjects without NAFLD (34).

Previously, aminotransferase levels and liver US imaging have been used to determine the epidemiology of NAFLD in adults and children, though NMRI provides better accuracy and aminotransferase levels can be normal in affected individuals. In our research, the aminotransferases were not an independent risk factor for NAFLD, though previously published results are conflicting. Guzzaloni et al., in a study that diagnosed moderate to severe NAFLD via US imaging for 375 obese children, found that liver enzymes were positively correlated with the level of NAFLD. In the study, abnormal liver enzyme levels were found in 48% of the children with NAFLD compared to 12% of those without the disease (35). Franzese et al., who evaluated the prevalence of NAFLD via US imaging in 72 obese children, showed that the number of cases of hypertransaminasemia was significantly higher for morbidly obese patients and those with a US diagnosis of serious NAFLD than for patients with moderate, mild or no NAFLD (7). More recently, Chan et al. found elevated aminotransferases in 29% of obese Chinese children with an US-based diagnosis of NAFLD and a strongly positive association between the seriousness of NAFLD and an elevation in aminotransferases (8). On the other hand, Browning et al. found that a high percentage (79%) of adults with NAFLD diagnosed via magnetic resonance spectroscopy had normal aminotransferase levels (36). Furthermore, using NMRI to diagnose NAFLD, Pacifico et al. demonstrated a lack of sensitivity when using blood aminotransferase levels to detect low levels of accumulation of liver fat (37).

Deivanayagem et al. demonstrated in overweight adolescents that high levels of inter-hepatic triglycerides, measured via NMRI with spectroscopy, are associated with increased insulin resistance, changes in glucose in the liver and

musculoskeletal system, and an abnormal lipid profile. These patients have a greater risk of developing type 2 diabetes and coronary artery disease and, for this reason, need to be monitored through regular medical check-ups and aggressive weight loss therapy (38).

Limitations

One of our study's limitations was the significantly greater presence of boys in the obese group (70.8%) compared to the eutrophic group (30.8%) ($p = 0.011$). Population studies have suggested that NAFLD is more common in boys than in girls and that this difference between the sexes is related to the severity of the lesion, which is more serious in males (24, 33). In this study, we did not observe an association between sex and NAFLD in the multivariate analysis. Obesity and AC were not determined to be independent factors for NAFLD in the multivariate analysis, despite expectations to the contrary. This result is likely due to the small number of patients analyzed.

A second limitation of this study was the small sample size compared to other pediatric studies that use only US imaging to diagnose NAFLD. However, because of the methodology used and the peculiarity of the study population, as indicated by the scarcity in scientific literature of studies using NMRIs to diagnose NAFLD in adolescents, it would have been difficult to evaluate a larger patient population sample during the study period. In fact, the number of patients studied was similar to that of other pediatric series evaluating the content of liver fat by magnetic resonance alone (15-17).

Another limitation in this study was the transverse design, which made it impossible to evaluate the progression of NAFLD and its relationship to

anthropometric and biochemical measures over time. In the future, a longitudinal study would provide a much more powerful data set for the analysis of the relationship between potential biomarkers and NAFLD. Additionally, liver biopsies could have aided us in confirming the FF calculated by NMRI and NAFLD. However, the goal of this study was to find non-invasive biomarkers.

A final limitation of the study is that this study evaluated insulin resistance based on the HOMA-IR calculation, which uses plasma glucose and basal insulin concentrations and thus is incapable of determining insulin's action in specific tissues, such as liver and muscle tissue (39, 40). HOMA uses a mathematical formula to adjust for individual variations in the secretion of insulin throughout the body and glucose liberation. While tests such as the euglycemic hyperinsulinemic clamp, the insulin suppression test, and the glucose tolerance blood test can determine insulin's action in specific tissues, these tests are costly and very difficult to apply in practice. Today, everyone agrees that HOMA, which is the most widely used measure of substitution in children, is highly correlated with fasting insulin ($r \geq 0.95$) in children (10) and in adults.

Conclusion

In conclusion, the HOMA-IR index is a predictive factor in diagnosing NAFLD, though there also appears to be an interaction between obesity level and HOMA-IR index. The potential use of HOMA-IR as a NAFLD diagnostic marker merits further testing, ideally in a longitudinal study with a larger study group.

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Table 1. NMRI accuracy in diagnosing mild, moderate and severe NAFLD, as described in a study by Pacifico L et al (21)

D. Table

Tables

Table 1. NMRI accuracy in diagnosing mild, moderate and severe NAFLD, as described in a study by Pacifico L et al(21).

Accuracy parameter	≥5% Grade I- mild	≥ 33% Grade II- moderate	≥ 66% Grade III- severe
Cutoff point	4.85	9	19
Area under the curve	0.98 (95%CI, 0.98-1.0)	1	1
Sensitivity%	95.8	100	100
Specificity%	100	100	100

Table 2. Clinical and laboratory characteristics of obese and eutrophic stufy groups.

Table 2. Clinical and laboratory characteristics of obese and eutrophic study groups.

Variables*	Eutrophic (n=26)	Obese (n=24)	P
Age (years)	14.7 ± 2.0	14.2 ± 1.9	0.379
Male	8 (30.8)	17 (70.8)	0.011
BMI (kg/m²)	21.1 ± 2.6	32.6 ± 3.8	<0.001
AC (cm)	4 (15.4)	23 (95.8)	<0.001
Glucose (mg/dl)	86.9 ± 8.4	94.3 ± 7.0	0.001
Insulin (µUI/ml)	6.0 (5.3–8.4)	14.9 (11.9–18.5)	<0.001
Total-C(mgl/dl)	165.7 ± 28.3	159.7 ± 25.0	0.435
HDL-C(mg/dl)	49.5 ± 9.7	39.7 ± 8.0	<0.001
Fibrinogen(g/l)	2.01 (1.78–2.37)	2.53 (1.93–3.00)	0.002
AlkalineP(U/L)	123.5 (96.3–107.3)	215.5 (111.5–295)	0.020
Gamma-GT (U/L)	18 (17–22)	27.5 (20–29)	0.011
C-reactive P (mg/l)	0.55 (0.2–1.6)	3.4 (1.08–5.45)	0.003
Oxaloacetic	16.5 (14–19.8)	20 (16.3–25.5)	0.011
Triglycerides (mg/dl)	60.5 (47–95)	89 (57.5–129.3)	0.103
Hepatic FF NMRI	2.95 (1.38–4.35)	7.10 (5.05–12.9)	<0.001
NAFLD	5 (19.2)	20 (83.3)	<0.001
HOMA IR	1.21 (1.10–1.89)	3.47 (2.83–4.37)	<0.001

* continuous variables described by average ± standard deviation or median (25-75 percentiles) and categorical variables by absolute and relative frequencies.

C-reactive P: high-sensitivity C-reactive Protein; HDL-C: high-density lipoprotein cholesterol; Total-C: total cholesterol; GOT: glumatic oxaloacetic transaminase, Gamma-GT: γ-glutamyl transpeptidase; AP: alkaline phosphatase; BMI: body mass index; AC: abdominal circumference.

Table 3. The clinical and laboratory characteristics of adolescents with and without NAFLD.

Table 3. The clinical and laboratory characteristics of adolescents with and withou NAFLD.

Variables*	**With NAFLD (n=25)**	**Without NAFLD (n=25)**	**P**
Age (years)	14.2 ± 2.0	14.8 ± 1.9	0.275
Male	14 (56.0)	11 (44.0)	0.572
BMI (Kg/m²)	31.1 ± 5.1	22.1 ± 4.6	<0.001
AC (cm)	21 (84.0)	6 (24.0)	<0.001
Glucose (mg/dl)	94.2 ± 6.2	86.6 ± 9.0	0.001
Insulin (µUI/ml)	15.2 (12.6–18.3)	5.9 (5.2–7.2)	<0.001
Total-C(mg/dl)	165.1 ± 23.2	160.7 ± 30.0	0.564
HDL-C(mg/dl)	41.3 ± 9.4	48.3 ± 9.8	0.014
Fibrinogen(g/l)	2.5 (2.0–2.95)	1.9 (1.75–2.33)	0.001
AlkalineP(U/L)	195 (112.5–295)	113 (93.5–227.5)	0.014
Gamma-GT (U/L)	26 (18–29)	20 (17.5–26)	0.139
C-reactive protein	2.2 (0.45–5.0)	0.7 (0.2–3.4)	0.113
Oxaloacetic	19 (15.5–23)	17 (14–22)	0.083
Triglycerides	88 (58 – 134.5)	60 (46–97)	0.042
HOMA IR	3.48 (2.87 – 4.34)	1.20 (1.09–1.48)	<0.001

* continuous variables described by average ± standard deviation or median (25-75 percentiles) and categorical variables by absolute and relative frequencies.

C-reactive P: high-sensitivity C-reactive Protein; HDL-C: high-density lipoprotein cholesterol; Total-C: total cholesterol; GOT: glumatic oxaloacetic transaminase, Gamma-GT: γ-glutamyl transpeptidase; AP: alkaline phosphatase; BMI: body mass index; AC: abdominal circumference.

Table 4. Multivariate Poisson regression to evaluate predictors independently associated with NAFLD.

Table 4. Multivariate Poisson regression to evaluate predictors independently associated with NAFLD.

Variables	Model 1		Model 2	
	PR (CI 95%)	P	PR (CI 95%)	P
Obese	1.47 (0.73–2.99)	0.283	NE	NE
High AC	NE	NE	1.91 (0.94–3.89)	0.074
Triglycerides	1.002 (1.000–1.004)	0.071	1.002 (1.000–1.005)	0.063
HOMA >2	13.7 (1.40–142.9)	0.024	13.0 (1.52–111)	0.019
HDL	1.01 (0.99–1.03)	0.157	1.01 (0.99–1.04)	0.214
Fibrinogen	1.12 (0.90–1.41)	0.316	1.08 (0.89–1.33)	0.436
AP	1.00 (0.99–1.00)	0.364	1.001 (0.99–1.003)	0.214
Amino-transferase	0.99 (0.98–1.01)	0.306	0.99 (0.97–1.00)	0.109

NE=not evaluated, AT=Aminotransferase
PR=Prevalence Ratios; CI 95%: 95% confidence intervals.

Figure 1. NMRI images of a patient with NAFLD, demonstrating the in-phase and out-of-phase signal intensity, the chemical shift, and the ROIs.

E. Figure

Figure 1. NMRI images of a patient with NAFLD, demonstrating the in-phase and out-of-phase signal intensity, the chemical shift, and the ROIs.

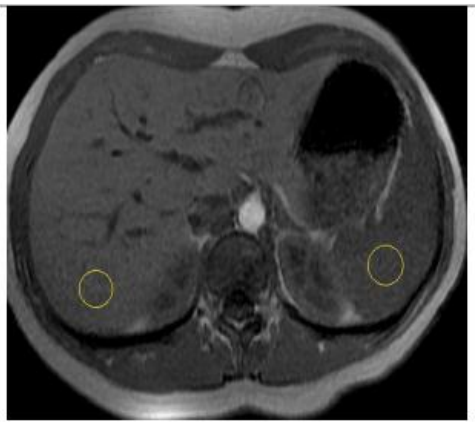
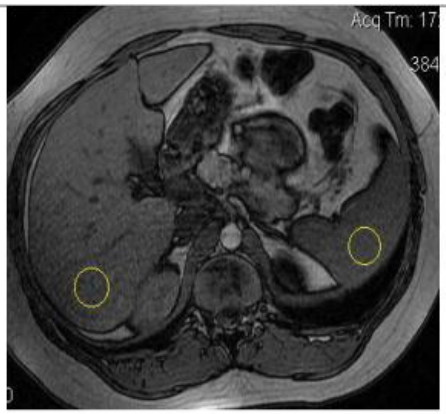
MRI in-phase	MRI out-of-phase
	
IP: 475,8	OP: 269,8
Fat fraction : 21,8%	NAFLD

Figure 2. NMRI images of a patient without NAFLD, demonstrating the in-phase and out-of-phase signal intensity, the chemical shift, and the ROIs.

Figure 2. NMRI images of a patient without NAFLD, demonstrating the in-phase and out-of-phase signal intensity, the chemical shift, and the ROIs.

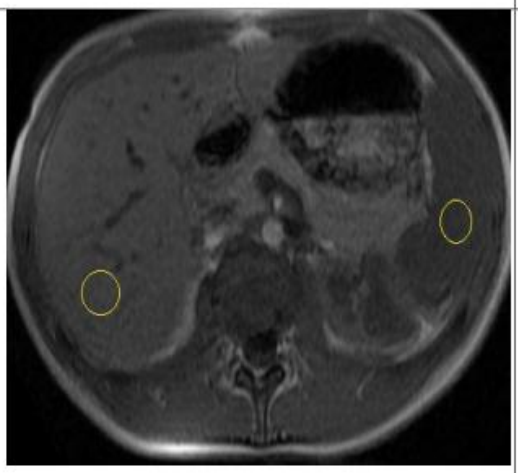
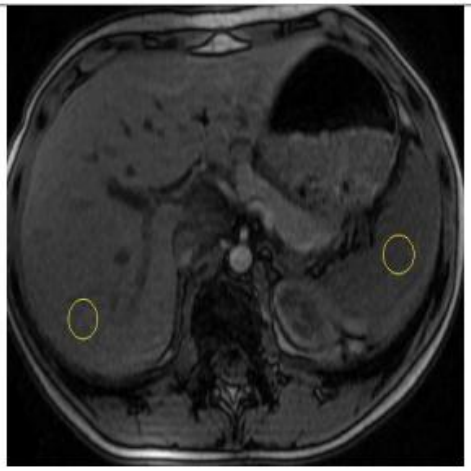
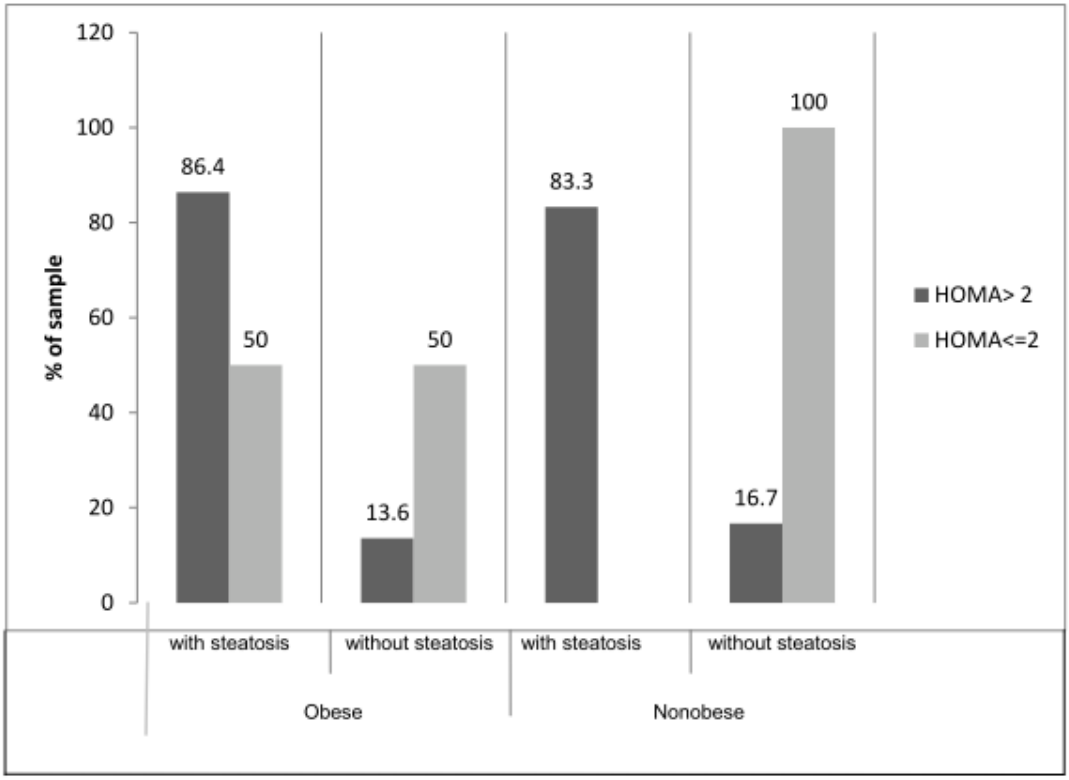
MRI in-phase	MRI out-phase
	
IS IP: 214,4	IS OP: 199,9
Fat fraction :1,4%	NORMAL

Figure 3. Evaluation of NAFLD in obese and eutrophic patients according to their HOMA classification.

Figure 3. Evaluation of NAFLD in obese and eutrophic patients according to their HOMA classification.



CAPÍTULO IV

CONCLUSÕES

4.1 CONCLUSÕES

O cálculo do GHRU, no diagnóstico da DHGNA, apresentou alta acurácia nos grupos de adolescentes estudados.

Sendo o US com a análise computadorizada com *Image J* e o cálculo do GHRU uma técnica simples para avaliação quantitativa da ecogenicidade hepática e não invasiva, ela poderá tornar-se um método diagnóstico importante no seguimento dos adolescentes obesos e eutróficos com DHGNA, considerando o reduzido tempo na realização do exame, a não exposição à radiação ionizante como na TC e os custos reduzidos em um contexto de saúde pública. Esse método, também, poderá servir para o rastreamento populacional da DHGNA, assim como para estudos clínicos.

No presente estudo, através da análise multivariada pode-se identificar que somente o HOMA-IR apresenta-se como um fator de risco independente de DHGNA. No entanto, parece haver uma interação entre obesidade e HOMA alterado, pois se HOMA-IR estiver alterado, a prevalência de esteatose é semelhante entre obesos e eutróficos. Se HOMA-IR estiver baixo, a prevalência de esteatose é maior em obesos do que em eutróficos. A prevalência de DHGNA em eutróficos, neste estudo, foi de 19,2% e em obesos de 83,3%.