Title: Resting Energy Expenditure of children with End Stage Chronic Liver Disease before and after liver transplantation.

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Contributions of authors:

Dr E Kyrana was involved in the study design, participant recruitment and measurements, data analysis and manuscript preparation.

Dr JE Williams was involved in the participant measurements and the data analysis.

Professor Wells was involved in the study design/methodology, data analysis and manuscript preparation.

Professor Dhawan was involved in the study design and manuscript preparation, as well as having the overall supervision of the work.

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Abstract:

Objectives:

Our objective was to test the hypothesis that children with end stage chronic liver disease (ESCLD) are hypermetabolic when compared to healthy children, and that this hypermetabolism persists for at least 6 months after liver transplant.

Methods:

17 patients with ESCLD and 14 healthy controls had their resting energy expenditure measured (mREE) by indirect calorimetry. Weight, height and BMI was converted to standard deviation (sd) scores. Children over 5 years had air displacement plethysmography (ADP) and patients over 5 years also had whole body DXA with characterization of fat mass (FM), fat free mass (FFM) and bone free fat free (LM).

Results:

When compared to the prediction equation 44% of the patients and 50% of the healthy controls were hypermetabolic. The younger patients (0-5 years) had a lower mREE than the healthy controls but were significantly lighter and shorter than their healthy counterparts. mREE correlated strongly for all children with age, weight, height and FFM. There was a strong negative correlation between age and mREE/kg in both patients ($r_s = -0.94$, $p < 0.01$) and controls ($r_s = -0.91$, $p < 0.01$). Almost 84% of the variance in mREE was explained by age ($p < 0.001$). There were no significant differences between REE/FFM between the 2 groups.

mREE/kg before liver transplant correlated with mREE/kg after transplant (Pearson's r = 0.83, p < 0.01).

Conclusions:

REE mostly reflected the size of the child. The patients were not hypermetabolic when compared to the healthy children. The main determinant of REE/kg after transplant was REE/kg before transplant.

What is known and what is new.

What is known:

- **Hypermetabolism has been frequently described in patients with end stage chronic liver disease.**
- **Hypermetabolism is not related to the severity of the liver disease.**
- **Resting energy expenditure in children after liver transplant has not been reported.**

What is new:

- **Resting energy expenditure was strongly associated with the size of the children.**
- **The patients with end stage chronic liver disease were not hypermetabolic when compared to the healthy controls.**
- **REE/kg before liver transplant was strongly associated to REE/kg after liver transplant.**

Introduction:

Hypermetabolism has frequently been described in patients with cirrhosis. It is usually defined as an increase of the resting energy expenditure (REE) of the patient to over 120% of the predicted value, as calculated by relevant equations or when compared to appropriately matched controls (1, 2). Among adults with cirrhosis, 15-31% are hypermetabolic (3, 4). Hypermetabolism is not associated with clinical severity or biochemical measures but has been linked to worse outcomes mainly before liver transplantation (4-6). Although fewer studies have looked at hypermetabolism after transplantation, they have shown a persistence of hypermetabolism after liver transplantation in adults, and an association with poorer outcomes (3, 5, 6).

Hypermetabolism in cirrhosis represents a maladaptation of the body to the reduction in caloric intake/ caloric absorption. These chronically ill patients differ from conditions such as starvation and anorexia nervosa, where humans tend to reduce their REE (7). Hypermetabolism will eventually lead to muscle loss and weight loss. Children with chronic liver disease have also been described as hypermetabolic (8, 9) .

This study aimed to test the hypothesis that children with ESCLD are hypermetabolic when compared to healthy children, and that this hypermetabolism persists for at least 6 months after liver transplant.

Methods:

To test these hypotheses, children with ESCLD waiting for a liver transplant were recruited to participate in a longitudinal observational study. Healthy children were also recruited to

match the patients for age and sex. The study received ethical approval from the London-Central REC (11/LO/1146).

All participants had their weight, height, mid upper arm circumference (MUAC)and their REE measured. Weight, height and BMI were converted to standard deviation (sd) scores with reference to UK-WHO data, using the ImsGrowth program© (10-12).

REE was quantified by indirect calorimetry using a metabolic monitor (Deltatrac II instrumentation; Datex-Ohmeda). The participants were measured at least 2 hours after any oral/ enteral intake. They were asked to lie down, and mixed expired gas was collected via a canopy hood for up to 30 minutes. The first 10 minutes of data were discarded and measured REE (mREE) was calculated as the average REE over the last 20 minutes (13, 14). mREE was recorded in Kcal/day and expressed as Kcal/day/kg of body weight. In addition, predicted REE (pREE) was calculated from the equations of Henry (15). Subsequently (mREE-pREE)/pREE *100% was calculated.

Children over the age of 5 years (patients and healthy controls) had air displacement plethysmography (ADP, Bodpod instrumentation; Life Measurement Instruments, Concord, CA). The younger children did not have ADP as it requires a certain degree of cooperation from them and therefore comparative reference data are not available. Two complete tests were performed on each child. Fat-free mass (FFM) and fat mass (FM) was calculated using published age- and sex-specific values for the density of fat-free tissue (16). Height-adjusted values for fat mass index (FMI) and fat free mass index (FFMI) were converted to sd scores using published UK reference data (16, 17).

Patients (but not healthy controls, because of the low irradiation dose) who were over the age of 5 years also had a whole body DXA scan. Comparative reference data for younger

children are not available. The DXA scanner used was the Lunar Prodigy Advance PA+ 303999 whole body scanner (GE Medical Systems, Madison, WI, with software Encore 2002). DXA provides data on total and regional body composition, including values of total FM, bone-free FFM (lean mass, LM) and bone mineral content (BMC). Data on FM and LM was expressed as sd scores to allow comparisons between the patients, using data from the UK reference populations previously mentioned (16, 17). FM and LM was also expressed as fat mass index (FMI) and lean mass index (LMI) and then in sd scores. This helps remove the effect of height, which is of relevance as paediatric patients often have short stature (18).

Baseline data on REE in the ESCLD patients were expressed as REE (mREE), as mREE/kg and, where possible, as mREE/KG of fat free mass (FFM). We also calculated predicted REE (pREE) as per the equations of Henry (2005). The same procedure was followed for the healthy children, and for the children with ESCLD who had a liver transplant at least 6 months after their liver transplant. Children with a measured REE of more than 120% of predicted REE were categorised as hypermetabolic. This was assessed for all 3 groups of children – patients at baseline, controls, and patients post liver transplant. Comparisons were made between the various groups (using unpaired t-test or Mann-Whitney U test if the sample did not follow the normal distribution). REE/kg of patients before and after liver transplantation was compared with a paired t-test. Associations of mREE with age, weight and height; mREE/kg with age; mREE with FFM and FM as by ADP; mREE with bone free LM and LMI as derived by DXA; and mREE/kg before and after liver transplantation were tested with Pearson correlation co-efficient or Spearman's correlation co-efficient, if one of the variables did not follow the normal distribution. IBM SPSS Statistics v24 was used for the statistical analysis. A p value of less than 0.05 was deemed statistically significant.

Results:

Seventeen patients (10F: 7M) with ESCLD were recruited. Age was 0.6 years to 17.2 years (mean 7.4 years, median 5 years). The diagnoses were biliary atresia (n=8), Alagille syndrome (n=2), neonatal sclerosing cholangitis (NSC) (n=1), a1-antitrypsin deficiency (n=1), primary sclerosing cholangitis (PSC) (n=1), cryptogenic cirrhosis (n=1) and hepatitis C virus (HCV) related cirrhosis (n=1) as well as two patients who were being assessed for a second transplant. Fourteen healthy controls (8F: 6M) also had body composition measurements. Their ages varied from 8 months to 18 years (mean 7.35 years, median 5.5 years).

The patients had significantly lower sd scores for weight and height compared to the controls. When the children were stratified by age group, these differences only persisted for the younger age group of 0-5 years (mean weight sds difference -1.99; 95%CI -2.99 to -1.08, p=0.0004 and mean height sds difference -2.47; 95%CI -3.45 to -1.49, p=0.0001). MUAC sd score was significantly lower in patients compared to controls (p< 0.01).

Body composition

Nine patients were old enough to have FM and FFM estimated by ADP, but only eight did, because one of them refused to enter the chamber (Table 1). Ten healthy controls also had ADP (Table 1).

Table 1: Indices from the Bodpod from the patients and the controls

All nine patients over the age of 5 years had a whole body DXA scan. All patients had FM and FMI sd scores either regional (arm, leg, trunk) or total, within normal limits; none were ≤ 1.96 sds. Mean sd scores for total LM were -0.74 (SD 1.00), arm LM -1.86 (SD 1.23), leg LM -1.55 (SD 1.00) and trunk LM 0.2 (SD 1.19). Mean sd scores for total LMI were -0.31 (SD

1.30), arm LMI -1.99 (SD 1.58), leg LMI -1.55 (SD 1.21) and trunk LMI 1.24 (SD 1.51). Five patients had appendicular LM sd scores ≤ 1.96 sds and three patients were sarcopenic with appendicular LMI sd scores ≤ 1.96 sds.

Indirect Calorimetry

REE before liver transplant

Seventeen children had their REE measured (mREE) prior to having a liver transplant with indirect calorimetry (Table 2). Valid results were obtained in sixteen, as one of the patients managed only 6 minutes under the hood.

Table 2: REE of patients before liver transplant and of healthy controls

Seven of the 16 children (44%) were categorised as hypermetabolic. None of the differences in mean sd score for weight, height, BMI and paediatric end stage liver disease (PELD) scores between the hypermetabolic and normometabolic group achieved statistical significance (Mann Whitney U test). The hypermetabolic patients in comparison to the normometabolic ones were not different in any of the liver function tests, including bilirubin levels.

We measured REE for thirteen healthy children and calculated their predicted REE. Only for twelve were the results valid (one child only managed 9 minutes and was restless) (Table 2). Six of the 12 (50%) healthy controls were categorised as hypermetabolic. None of the differences of the mean sd scores for weight, height or BMI between the hypermetabolic and normometabolic group achieved statistical significance (Mann Whitney U test).

When comparing the patients with healthy controls, there was no significant difference in mean age or mean mREE/kg BW (Mann Whitney U test). Seven of the patients could be

paired for sex and age; the paired differences for REE/kg were close to zero and far from significant.

There was a strong negative correlation between age and mREE/kg in both the patients (Spearman's r_s = -0.94, p <0.01) and the controls (Spearman's r_s= -0.91, p<0.01). Almost 84% of the variance in mREE could be explained by age (p <0.001). The patients had a tendency for a lower mREE than the controls and the younger they were, the stronger the tendency (Figure 1). There was a strong positive correlation of mREE with weight and height for the patients and the healthy controls (Spearman's r_s 0.938 and 0.946 respectively for the patients, p<0.01, and 0.811 and 0.804, p< 0.05 for the controls).

When we divided the patients and the healthy controls into 3 age groups: 0-5 years, 5-10 years and over 10 years of age, we found that the mean mREE was significantly lower than that of their healthy counterparts only in the younger group (mean 638.15 Kcal versus 1076 Kcal, 95%CI -706.1 to -169.66 Kcal; p<0.005), and not in the other groups [for the 5-10 year olds mean mREE 1312.75 Kcal versus 1219 Kcal, 95%CI -134.31 to 321.81 Kcal and for the over 10 year olds mean mREE 1630.8 Kcal versus 1440 Kcal, 95%CI -72.53 to 454.13 Kcal) (Figure 1**).**

Figure 1: mREE of patients before and after liver transplant and of healthy controls over different age groups

Mean mREE/kg was greater in patients than healthy children in the age group 5-10 years (mean REE/kg 53.04 Kcal/kg versus 43.48, Δ= 9.92, 95%CI 3.0 to 16.1, p<0.05), but not in the younger or older children (< 5 years patients versus healthy children mean REE/kg 72.6 Kcal/kg versus 72.1 Kcal/kg, Δ= 0.5, 95%CI -11.5 to 16.3 and for the over 10 years of age mean REE/kg was 34.8 Kcal/kg versus 31.6 Kcal/kg, Δ= 3.2, 95%CI -11.2, 17.4). For these

same groups there were no significant differences between mean mREE, between weight, weight sds or between mREE/FFM as measured by Bodpod.

REE and body composition

There was no significant difference between mean mREE/FFM of patients and healthy controls overall and within different age groups (unpaired t-test). Mean mREE/FFM was not statistically different between the patients 5-10 years of age and the healthy controls of the same age. Mean mREE/FFM between the hypermetabolic and the normo-metabolic patients was not significantly different, neither was mean mREE/FFM between the hypermetabolic and normo-metabolic healthy controls (unpaired t-test). Three of the patients had a FFM sds ≤ 1.96. mREE/FFM for these 3 patients was not significantly different to the mREE/FFM of the rest of the patients (unpaired t-test). The five patients with the very low appendicular LM sd scores by DXA, when compared with the rest of the patients, did not have a significantly different mREE/LM (unpaired t-test).

For the patients and the healthy children, mREE correlated strongly with FFM, as derived from the BOD POD (Spearman's rho = 0.785 and 0.763; p=0.001 and <0.0001 respectively) and not with FM.

REE after liver transplant

Ten of the patients had REE re-assessed post liver transplant. The repeat measurements were done on average 16.9 months after the first body composition assessment (median 16 months, range 10.8 to 29.2 months) and on average 10.8 months after the liver transplant (median 9.8 months, range 6 to 19.9 months). Of these 10 patients, 3 were hypermetabolic prior to their liver transplant when comparing to prediction equations. They remained

hypermetabolic after the transplant, while an additional 4 more became hypermetabolic after the transplant (7/10).

The differences in mean REE/kg BW before versus after liver transplantation were not statistically significant (unpaired t-test). Differences in REE/kg were not related to mortality or outcomes like length of stay in intensive care, length of stay in hospital post-transplant, vascular complications, infections and post-transplant lymphoproliferative disorder. At the time of the repeat assessments, all transplanted children were well in themselves; they were outpatients, and all had normal liver function, apart from one patient who subsequently was found to have acute cellular rejection the next day. mREE/kg before liver transplant correlated with mREE/kg after liver transplant (Pearson's r = 0.83, p < 0.01) and as did mREE/kg of bone free lean mass (for the 5 patients that had DXA scans) (Pearson's r = 0.928, p<0.05) (Figure 2).

Figure 2: Correlation between mREE/kg in kcal/kg before and after liver transplantation

Discussion:

In this study we measured REE of children with ESCLD before liver transplant and compared it to their predicted REE as well as to that of healthy children. We also measured REE of some of the patients after their liver transplant. When compared to the prediction equations, 44% of the patients were hypermetabolic at baseline, as were 50% of the healthy children. The equations used appear to have underestimated REE. This could be due to differences between our study population and the cohorts used to generate the equations, or to differences in the methodology used to measure the REE. An advantage in this study was that measurements were also obtained in healthy controls and were done following the same protocol and by the same person, and therefore systematic error could be minimised.

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Expressing REE per kg of weight helps compare between the different patients. None of the patients had overt ascites, nevertheless weight in chronic liver disease tends to be overestimated as there is a degree of fluid retention. Differences in mREE/kg were not related to outcomes. mREE/kg before liver transplant was a strong predictor of mREE/kg after liver transplant. This has been shown in adult patients with cirrhosis. It implies that mREE/kg depends more on idiosyncratic characteristics of the patients (age, genetics etc.) rather than the disease itself (3). mREE/kg of LM before the transplant was also strongly associated with mREE/kg of LM after the transplant.

Frequently REE is expressed per kg FFM, as this component of the body is considered the most metabolically active. There are however some inherent problems with this approach. FFM includes bone, organs and muscle and these elements have a very different metabolic profile and very different energy consumption e.g. muscle contributes 20% to REE whereas the organs 60%. Patients with liver disease are likely to have a different composition of their FFM, which may not allow a direct comparison with someone healthy. The relationship between FFM and REE is not linear for the full range of FFM (7). In addition, measuring FFM comes with its own challenges and inaccuracies. Our measurements confirmed a strong correlation of REE with FFM, but we were not able to demonstrate significant differences between REE/kg FFM between the patients and healthy children.

The few studies that report on REE of children with chronic liver disease (8, 9), claim that overall children with chronic liver disease are hypermetabolic. Our study shows that there is a high degree of variation in REE when it is measured, rather than predicted, and not all patients can be classified as hypermetabolic. This is consistent with findings in children with intestinal failure related liver disease (19). Classifying children as hypermetabolic based on

definitions used for adults may not be that helpful. The changes in metabolic rate are on a spectrum and are most likely linked more to idiosyncratic features of the child. Our data did not show an association between hypermetabolism post liver transplant and worse liver function. When comparing to the equations, there is a noticeable increase in the number of patients categorised hypermetabolic post liver transplant, but there was not an increase in REE/kg weight or per kg of lean mass. This suggests that the equations have an age bias in the likelihood of being categorised hypermetabolic.

Our data show that the patients younger than 5 years had lower measured REE than the healthy children. This could reflect their smaller size and indeed when adjusted for weight this difference vanishes. Children with chronic liver disease transplanted before the age of 5 years tend to have more significant nutritional and growth issues than the ones brought to transplant at an older age. Our data showed a strong correlation between REE and age of the child and it did not support the presence of a higher REE in the patients in comparison to the healthy children, except for the children who were 5-10 years of age. They had a higher REE/kg body weight than their healthy counterparts, without significant differences in the age within the group, their weight or FFM. As there was no significant difference when comparing the REE/kg of FFM within that age group, the differences found may be due to idiosyncratic features.

Very few studies have investigated energy requirements of children with chronic liver disease and no other studies, to our knowledge, have investigated energy requirements of these children post liver transplant. Our study favours measuring individually energy requirements for the patients that require intense nutritional management, as reported by Carpenter et al (20), rather than assuming hypermetabolism. Resting energy requirements

are only one aspect of a tailored nutritional plan, as of course one would need to account for other aspects relevant to these patients like fat malabsorption. In children, growth is the best indicator we have that nutritional needs are being met. Growth though is also influenced by other factors like the presence of inflammation, insulin resistance and medicines, like steroids. Aggressive nutritional management is at the core of chronic liver disease management and has been repeatedly shown to reduce progression to ESCLD and to improve liver transplant outcomes (21). What is also important is nutritional management after liver transplantation. Studies have shown a growth spurt in the first 2 years after transplant (22-25), which would increase the child's nutrition requirements. Whereas most children would naturally facilitate for this, others many need support. We have entered an era where our nutritional management needs to be more individualised and sophisticated and based on research that can address the longer term outcomes of our patients, outcomes including cardiovascular risk and neurocognition. Steroid use is common practice after Kasai portoenterostomy in biliary atresia, and standard practice after liver transplant. It has been shown to influence height achieved post liver transplant (22, 26) and we know steroids influence metabolism and muscle mass, but we do not know the impact of these treatments for the children after liver transplant. Post liver transplant, measurements of REE for longer periods after the transplant and correlation with body composition would be of value, particularly as reports of obesity, sarcopenia and metabolic syndrome post solid organ transplant are increasing.

In summary, prediction of REE with the Henry 2005 equations underestimates REE for patients and healthy controls. REE correlated strongly for all children with age, weight, height and FFM. The younger patients had a lower REE than the healthy controls and this difference tended to be bigger the younger they were. These younger patients were lighter

and shorter than their healthy counterparts. FM and FMI as assessed by ADP and DXA was within normal limits for all patients. Some patients were sarcopenic (in spite of normal FM and FMI) with low FFM and FFMI, but there were no significant differences between REE/FFM between patients and healthy controls. REE mostly reflected the size of the child. REE/kg reduced with increasing age. The main determinant of REE/kg after transplant was REE/kg before transplant.

Legends

Table 1

Bodpod air displacement plethysmography, Age in years, M male, F female, sds standard deviation score, FM fat mass, FFM fat free mass, TBD total body density

All the patients had a FM sd score within normal limits, but 3 of the patients were sarcopenic (sd score ≤ 1.96). One healthy child had a low fat and lean mass sd score.

Table 2

F female, M male, kg kilograms, cm centimetres, kcal kilocalorie, mREE measured resting energy expenditure, pREE predicted resting energy expenditure, BW body weight, in bold the measurements of REE that over 20% the predicted REE.

Figure 1

REE_kg Resting energy expenditure per kilogram; mREE measured resting energy expenditure; OLT orthotopic liver transplantation

Figure 2

mREE/kg Post Ltx resting energy expenditure per kilogram of body weight after liver transplantation

mREE/kg/ Pre Ltx resting energy expenditure per kilogram of body weight before liver transplantation

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Conclusions:

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What is known and what is new.

What is known:

- **Hypermetabolism has been frequently described in patients with end stage chronic liver disease.**
- **Hypermetabolism is not related to the severity of the liver disease.**
- **Resting energy expenditure in children after liver transplant has not been reported.**

What is new:

- **Resting energy expenditure was strongly associated with the size of the children.**
- **The patients with end stage chronic liver disease were not hypermetabolic when compared to the healthy controls.**
- **REE/kg before liver transplant was strongly associated to REE/kg after liver transplant.**

Introduction:

Hypermetabolism has frequently been described in patients with cirrhosis. It is usually defined as an increase of the resting energy expenditure (REE) of the patient to over 120% of the predicted value, as calculated by relevant equations or when compared to appropriately matched controls (1, 2). Among adults with cirrhosis, 15-31% are hypermetabolic (3, 4). Hypermetabolism is not associated with clinical severity or biochemical measures but has been linked to worse outcomes mainly before liver transplantation (4-6). Although fewer studies have looked at hypermetabolism after transplantation, they have shown a persistence of hypermetabolism after liver transplantation in adults, and an association with poorer outcomes (3, 5, 6).

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Methods:

To test these hypotheses, children with ESCLD waiting for a liver transplant were recruited to participate in a longitudinal observational study. Healthy children were also recruited to

match the patients for age and sex. The study received ethical approval from the London-Central REC (11/LO/1146).

All participants had their weight, height, mid upper arm circumference (MUAC)and their REE measured. Weight, height and BMI were converted to standard deviation (sd) scores with reference to UK-WHO data, using the ImsGrowth program© (10-12).

REE was quantified by indirect calorimetry using a metabolic monitor (Deltatrac II instrumentation; Datex-Ohmeda). The participants were measured at least 2 hours after any oral/ enteral intake. They were asked to lie down, and mixed expired gas was collected via a canopy hood for up to 30 minutes. The first 10 minutes of data were discarded and measured REE (mREE) was calculated as the average REE over the last 20 minutes (13, 14). mREE was recorded in Kcal/day and expressed as Kcal/day/kg of body weight. In addition, predicted REE (pREE) was calculated from the equations of Henry (15). Subsequently (mREE-pREE)/pREE *100% was calculated.

Children over the age of 5 years (patients and healthy controls) had air displacement plethysmography (ADP, Bodpod instrumentation; Life Measurement Instruments, Concord, CA). The younger children did not have ADP as it requires a certain degree of cooperation from them and therefore comparative reference data are not available. Two complete tests were performed on each child. Fat-free mass (FFM) and fat mass (FM) was calculated using published age- and sex-specific values for the density of fat-free tissue (16). Height-adjusted values for fat mass index (FMI) and fat free mass index (FFMI) were converted to sd scores using published UK reference data (16, 17).

Patients (but not healthy controls, because of the low irradiation dose) who were over the age of 5 years also had a whole body DXA scan. Comparative reference data for younger

children are not available. The DXA scanner used was the Lunar Prodigy Advance PA+ 303999 whole body scanner (GE Medical Systems, Madison, WI, with software Encore 2002). DXA provides data on total and regional body composition, including values of total FM, bone-free FFM (lean mass, LM) and bone mineral content (BMC). Data on FM and LM was expressed as sd scores to allow comparisons between the patients, using data from the UK reference populations previously mentioned (16, 17). FM and LM was also expressed as fat mass index (FMI) and lean mass index (LMI) and then in sd scores. This helps remove the effect of height, which is of relevance as paediatric patients often have short stature (18).

Baseline data on REE in the ESCLD patients were expressed as REE (mREE), as mREE/kg and, where possible, as mREE/KG of fat free mass (FFM). We also calculated predicted REE (pREE) as per the equations of Henry (2005). The same procedure was followed for the healthy children, and for the children with ESCLD who had a liver transplant at least 6 months after their liver transplant. Children with a measured REE of more than 120% of predicted REE were categorised as hypermetabolic. This was assessed for all 3 groups of children – patients at baseline, controls, and patients post liver transplant. Comparisons were made between the various groups (using unpaired t-test or Mann-Whitney U test if the sample did not follow the normal distribution). REE/kg of patients before and after liver transplantation was compared with a paired t-test. Associations of mREE with age, weight and height; mREE/kg with age; mREE with FFM and FM as by ADP; mREE with bone free LM and LMI as derived by DXA; and mREE/kg before and after liver transplantation were tested with Pearson correlation co-efficient or Spearman's correlation co-efficient, if one of the variables did not follow the normal distribution. IBM SPSS Statistics v24 was used for the statistical analysis. A p value of less than 0.05 was deemed statistically significant.

Results:

Seventeen patients (10F: 7M) with ESCLD were recruited. Age was 0.6 years to 17.2 years (mean 7.4 years, median 5 years). The diagnoses were biliary atresia (n=8), Alagille syndrome (n=2), neonatal sclerosing cholangitis (NSC) (n=1), a1-antitrypsin deficiency (n=1), primary sclerosing cholangitis (PSC) (n=1), cryptogenic cirrhosis (n=1) and hepatitis C virus (HCV) related cirrhosis (n=1) as well as two patients who were being assessed for a second transplant. Fourteen healthy controls (8F: 6M) also had body composition measurements. Their ages varied from 8 months to 18 years (mean 7.35 years, median 5.5 years).

The patients had significantly lower sd scores for weight and height compared to the controls. When the children were stratified by age group, these differences only persisted for the younger age group of 0-5 years (mean weight sds difference -1.99; 95%CI -2.99 to -1.08, p=0.0004 and mean height sds difference -2.47; 95%CI -3.45 to -1.49, p=0.0001). MUAC sd score was significantly lower in patients compared to controls (p< 0.01).

Body composition

Nine patients were old enough to have FM and FFM estimated by ADP, but only eight did, because one of them refused to enter the chamber (Table 1). Ten healthy controls also had ADP (Table 1).

Table 1: Indices from the Bodpod from the patients and the controls

All nine patients over the age of 5 years had a whole body DXA scan. All patients had FM and FMI sd scores either regional (arm, leg, trunk) or total, within normal limits; none were ≤ 1.96 sds. Mean sd scores for total LM were -0.74 (SD 1.00), arm LM -1.86 (SD 1.23), leg LM -1.55 (SD 1.00) and trunk LM 0.2 (SD 1.19). Mean sd scores for total LMI were -0.31 (SD

1.30), arm LMI -1.99 (SD 1.58), leg LMI -1.55 (SD 1.21) and trunk LMI 1.24 (SD 1.51). Five patients had appendicular LM sd scores ≤ 1.96 sds and three patients were sarcopenic with appendicular LMI sd scores ≤ 1.96 sds.

Indirect Calorimetry

REE before liver transplant

Seventeen children had their REE measured (mREE) prior to having a liver transplant with indirect calorimetry (Table 2). Valid results were obtained in sixteen, as one of the patients managed only 6 minutes under the hood.

Table 2: REE of patients before liver transplant and of healthy controls

Seven of the 16 children (44%) were categorised as hypermetabolic. None of the differences in mean sd score for weight, height, BMI and paediatric end stage liver disease (PELD) scores between the hypermetabolic and normometabolic group achieved statistical significance (Mann Whitney U test). The hypermetabolic patients in comparison to the normometabolic ones were not different in any of the liver function tests, including bilirubin levels.

We measured REE for thirteen healthy children and calculated their predicted REE. Only for twelve were the results valid (one child only managed 9 minutes and was restless) (Table 2). Six of the 12 (50%) healthy controls were categorised as hypermetabolic. None of the differences of the mean sd scores for weight, height or BMI between the hypermetabolic and normometabolic group achieved statistical significance (Mann Whitney U test).

When comparing the patients with healthy controls, there was no significant difference in mean age or mean mREE/kg BW (Mann Whitney U test). Seven of the patients could be

paired for sex and age; the paired differences for REE/kg were close to zero and far from significant.

There was a strong negative correlation between age and mREE/kg in both the patients (Spearman's r_s = -0.94, p <0.01) and the controls (Spearman's r_s= -0.91, p<0.01). Almost 84% of the variance in mREE could be explained by age (p <0.001). The patients had a tendency for a lower mREE than the controls and the younger they were, the stronger the tendency (Figure 1). There was a strong positive correlation of mREE with weight and height for the patients and the healthy controls (Spearman's r_s 0.938 and 0.946 respectively for the patients, p<0.01, and 0.811 and 0.804, p< 0.05 for the controls).

When we divided the patients and the healthy controls into 3 age groups: 0-5 years, 5-10 years and over 10 years of age, we found that the mean mREE was significantly lower than that of their healthy counterparts only in the younger group (mean 638.15 Kcal versus 1076 Kcal, 95%CI -706.1 to -169.66 Kcal; p<0.005), and not in the other groups [for the 5-10 year olds mean mREE 1312.75 Kcal versus 1219 Kcal, 95%CI -134.31 to 321.81 Kcal and for the over 10 year olds mean mREE 1630.8 Kcal versus 1440 Kcal, 95%CI -72.53 to 454.13 Kcal) (Figure 1**).**

Figure 1: mREE of patients before and after liver transplant and of healthy controls over different age groups

Mean mREE/kg was greater in patients than healthy children in the age group 5-10 years (mean REE/kg 53.04 Kcal/kg versus 43.48, Δ= 9.92, 95%CI 3.0 to 16.1, p<0.05), but not in the younger or older children (< 5 years patients versus healthy children mean REE/kg 72.6 Kcal/kg versus 72.1 Kcal/kg, Δ= 0.5, 95%CI -11.5 to 16.3 and for the over 10 years of age mean REE/kg was 34.8 Kcal/kg versus 31.6 Kcal/kg, Δ= 3.2, 95%CI -11.2, 17.4). For these

same groups there were no significant differences between mean mREE, between weight, weight sds or between mREE/FFM as measured by Bodpod.

REE and body composition

There was no significant difference between mean mREE/FFM of patients and healthy controls overall and within different age groups (unpaired t-test). Mean mREE/FFM was not statistically different between the patients 5-10 years of age and the healthy controls of the same age. Mean mREE/FFM between the hypermetabolic and the normo-metabolic patients was not significantly different, neither was mean mREE/FFM between the hypermetabolic and normo-metabolic healthy controls (unpaired t-test). Three of the patients had a FFM sds ≤ 1.96. mREE/FFM for these 3 patients was not significantly different to the mREE/FFM of the rest of the patients (unpaired t-test). The five patients with the very low appendicular LM sd scores by DXA, when compared with the rest of the patients, did not have a significantly different mREE/LM (unpaired t-test).

For the patients and the healthy children, mREE correlated strongly with FFM, as derived from the BOD POD (Spearman's rho = 0.785 and 0.763; p=0.001 and <0.0001 respectively) and not with FM.

REE after liver transplant

Ten of the patients had REE re-assessed post liver transplant. The repeat measurements were done on average 16.9 months after the first body composition assessment (median 16 months, range 10.8 to 29.2 months) and on average 10.8 months after the liver transplant (median 9.8 months, range 6 to 19.9 months). Of these 10 patients, 3 were hypermetabolic prior to their liver transplant when comparing to prediction equations. They remained

hypermetabolic after the transplant, while an additional 4 more became hypermetabolic after the transplant (7/10).

The differences in mean REE/kg BW before versus after liver transplantation were not statistically significant (unpaired t-test). Differences in REE/kg were not related to mortality or outcomes like length of stay in intensive care, length of stay in hospital post-transplant, vascular complications, infections and post-transplant lymphoproliferative disorder. At the time of the repeat assessments, all transplanted children were well in themselves; they were outpatients, and all had normal liver function, apart from one patient who subsequently was found to have acute cellular rejection the next day. mREE/kg before liver transplant correlated with mREE/kg after liver transplant (Pearson's r = 0.83, p < 0.01) and as did mREE/kg of bone free lean mass (for the 5 patients that had DXA scans) (Pearson's r = 0.928, p<0.05) (Figure 2).

Figure 2: Correlation between mREE/kg in kcal/kg before and after liver transplantation

Discussion:

In this study we measured REE of children with ESCLD before liver transplant and compared it to their predicted REE as well as to that of healthy children. We also measured REE of some of the patients after their liver transplant. When compared to the prediction equations, 44% of the patients were hypermetabolic at baseline, as were 50% of the healthy children. The equations used appear to have underestimated REE. This could be due to differences between our study population and the cohorts used to generate the equations, or to differences in the methodology used to measure the REE. An advantage in this study was that measurements were also obtained in healthy controls and were done following the same protocol and by the same person, and therefore systematic error could be minimised.

Expressing REE per kg of weight helps compare between the different patients. None of the patients had overt ascites, nevertheless weight in chronic liver disease tends to be overestimated as there is a degree of fluid retention. Differences in mREE/kg were not related to outcomes. mREE/kg before liver transplant was a strong predictor of mREE/kg after liver transplant. This has been shown in adult patients with cirrhosis. It implies that mREE/kg depends more on idiosyncratic characteristics of the patients (age, genetics etc.) rather than the disease itself (3). mREE/kg of LM before the transplant was also strongly associated with mREE/kg of LM after the transplant.

Frequently REE is expressed per kg FFM, as this component of the body is considered the most metabolically active. There are however some inherent problems with this approach. FFM includes bone, organs and muscle and these elements have a very different metabolic profile and very different energy consumption e.g. muscle contributes 20% to REE whereas the organs 60%. Patients with liver disease are likely to have a different composition of their FFM, which may not allow a direct comparison with someone healthy. The relationship between FFM and REE is not linear for the full range of FFM (7). In addition, measuring FFM comes with its own challenges and inaccuracies. Our measurements confirmed a strong correlation of REE with FFM, but we were not able to demonstrate significant differences between REE/kg FFM between the patients and healthy children.

The few studies that report on REE of children with chronic liver disease (8, 9), claim that overall children with chronic liver disease are hypermetabolic. Our study shows that there is a high degree of variation in REE when it is measured, rather than predicted, and not all patients can be classified as hypermetabolic. This is consistent with findings in children with intestinal failure related liver disease (19). Classifying children as hypermetabolic based on

definitions used for adults may not be that helpful. The changes in metabolic rate are on a spectrum and are most likely linked more to idiosyncratic features of the child. Our data did not show an association between hypermetabolism post liver transplant and worse liver function. When comparing to the equations, there is a noticeable increase in the number of patients categorised hypermetabolic post liver transplant, but there was not an increase in REE/kg weight or per kg of lean mass. This suggests that the equations have an age bias in the likelihood of being categorised hypermetabolic.

Our data show that the patients younger than 5 years had lower measured REE than the healthy children. This could reflect their smaller size and indeed when adjusted for weight this difference vanishes. Children with chronic liver disease transplanted before the age of 5 years tend to have more significant nutritional and growth issues than the ones brought to transplant at an older age. Our data showed a strong correlation between REE and age of the child and it did not support the presence of a higher REE in the patients in comparison to the healthy children, except for the children who were 5-10 years of age. They had a higher REE/kg body weight than their healthy counterparts, without significant differences in the age within the group, their weight or FFM. As there was no significant difference when comparing the REE/kg of FFM within that age group, the differences found may be due to idiosyncratic features.

Very few studies have investigated energy requirements of children with chronic liver disease and no other studies, to our knowledge, have investigated energy requirements of these children post liver transplant. Our study favours measuring individually energy requirements for the patients that require intense nutritional management, as reported by Carpenter et al (20), rather than assuming hypermetabolism. Resting energy requirements

are only one aspect of a tailored nutritional plan, as of course one would need to account for other aspects relevant to these patients like fat malabsorption. In children, growth is the best indicator we have that nutritional needs are being met. Growth though is also influenced by other factors like the presence of inflammation, insulin resistance and medicines, like steroids. Aggressive nutritional management is at the core of chronic liver disease management and has been repeatedly shown to reduce progression to ESCLD and to improve liver transplant outcomes (21). What is also important is nutritional management after liver transplantation. Studies have shown a growth spurt in the first 2 years after transplant (22-25), which would increase the child's nutrition requirements. Whereas most children would naturally facilitate for this, others many need support. We have entered an era where our nutritional management needs to be more individualised and sophisticated and based on research that can address the longer term outcomes of our patients, outcomes including cardiovascular risk and neurocognition. Steroid use is common practice after Kasai portoenterostomy in biliary atresia, and standard practice after liver transplant. It has been shown to influence height achieved post liver transplant (22, 26) and we know steroids influence metabolism and muscle mass, but we do not know the impact of these treatments for the children after liver transplant. Post liver transplant, measurements of REE for longer periods after the transplant and correlation with body composition would be of value, particularly as reports of obesity, sarcopenia and metabolic syndrome post solid organ transplant are increasing.

In summary, prediction of REE with the Henry 2005 equations underestimates REE for patients and healthy controls. REE correlated strongly for all children with age, weight, height and FFM. The younger patients had a lower REE than the healthy controls and this difference tended to be bigger the younger they were. These younger patients were lighter

and shorter than their healthy counterparts. FM and FMI as assessed by ADP and DXA was within normal limits for all patients. Some patients were sarcopenic (in spite of normal FM and FMI) with low FFM and FFMI, but there were no significant differences between REE/FFM between patients and healthy controls. REE mostly reflected the size of the child. REE/kg reduced with increasing age. The main determinant of REE/kg after transplant was REE/kg before transplant.

Legends

Table 1

Bodpod air displacement plethysmography, Age in years, M male, F female, sds standard deviation score, FM fat mass, FFM fat free mass, TBD total body density

All the patients had a FM sd score within normal limits, but 3 of the patients were sarcopenic (sd score ≤ 1.96). One healthy child had a low fat and lean mass sd score.

Table 2

F female, M male, kg kilograms, cm centimetres, kcal kilocalorie, mREE measured resting energy expenditure, pREE predicted resting energy expenditure, BW body weight, in bold the measurements of REE that over 20% the predicted REE.

Figure 1

REE_kg Resting energy expenditure per kilogram; mREE measured resting energy expenditure; OLT orthotopic liver transplantation

Figure 2

mREE/kg Post Ltx resting energy expenditure per kilogram of body weight after liver transplantation

mREE/kg/ Pre Ltx resting energy expenditure per kilogram of body weight before liver transplantation

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Figure 1

Figure 1: mREE of patients before and after liver transplant and of healthy controls over different age groups
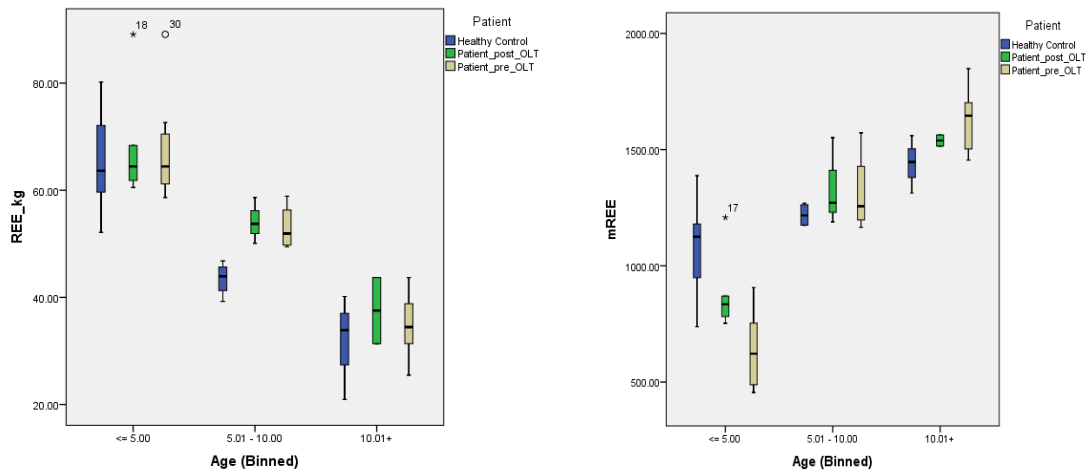
REE_kg Resting energy expenditure per kilogram; mREE measured resting energy expenditure; OLT orthotopic liver transplantation

Figure 2

Figure.1: Correlation between mREE/kg in kcal/kg before and after liver transplantation
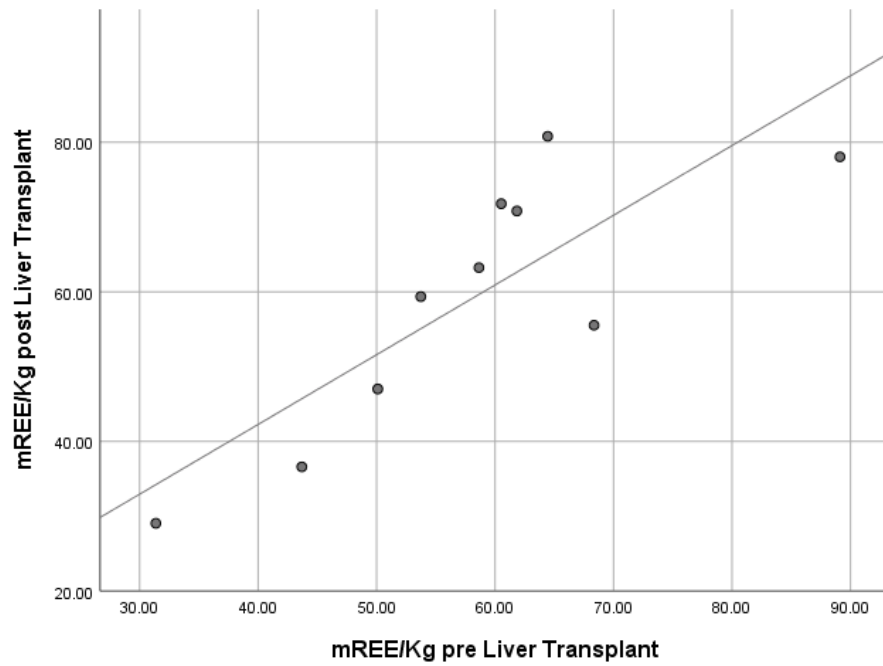
mREE/kg Post Ltx resting energy expenditure per kilogram of body weight after liver transplantation

mREE/kg/ Pre Ltx resting energy expenditure per kilogram of body weight before liver transplantation

Table 1

Table 1: Indices from the Bodpod from the patients and the healthy controls

Bodpod indices from the patients				
Age	**Sex**	**FM%**	**FM sds**	**FFM sds**
9.9	F	17.9	-0.87	0.14
5.2	M	24.8	1.10	-0.15
10.5	F	24.3	-0.30	-0.58
9.3	M	14.6	-1.14	**-1.96**
17.2	M	34.3	0.91	**-3.22**
16.7	F	34.9	0.66	-0.88
8.7	F	32.7	0.12	**-2.32**
Bodpod indices from the healthy controls				
Age	**Sex**	**FM%**	**FM sds**	**FFM sds**
4.6	M	12.9	-0.33	0.68
4.5	M	20.7	0.57	-0.72
10.1	M	16.2	-0.48	-0.53
13.5	F	26.3	-0.21	-1.26
9.7	M	28.6	0.86	-0.66
17.9	F	33.9	0.81	0.54
15.8	M	6.0	**-2.53**	**-2.45**
4.3	F	25.1	0.65	0.73
6.5	F	25.4	0.3	0.32
9.6	F	27.3	-0.32	-1.86

Bodpod air displacement plethysmography, Age in years, M male, F female, sds standard deviation score, FM fat mass, FFM fat free mass, TBD total body density

All the patients had a FM sd score within normal limits, but 3 of the patients were sarcopenic (sd score ≤ 1.96). One healthy child had a low fat and lean mass sd score.

Table 2

Table Error! No text of specified style in document.**-2: REE of patients before liver transplant and of healthy controls**

REE of patients before liver transplant							
Age (years)	Sex	Weight (kg)	Height (cm)	mREE (kcal)	pREE (kcal)	(mREE-pREE) /pREE*100%	mREE/ kg BW
9.9	F	31.8	154.6	1572	1179	**33.3**	49.43
1.76	M	11.04	80.2	802	629	**27.5**	72.64
5.2	M	21.7	112.4	1166	717	**62.6**	53.73
0.6	F	7.14	68	488	408	19.6	68.35
4.7	F	15.45	99.25	906	803	12.8	58.64
0.8	M	8.08	68	489	441	10.9	60.52
3.0	F	11.4	89.9	705	719	-0.02	61.84
0.7	F	6.98	65.9	622	388	**60.3**	89.11
10.5	F	33.3	131.2	1455	1102	**32**	43.69
1.0	F	7.06	68.1	455	406	12.1	64.44
15.1	F	42.4	138	1646	1204	**36.7**	38.82
9.3	M	21.8	119.6	1284	724	**77.3**	58.90
17.2	M	54.26	159.6	1702	1570	8.4	31.37
16.7	F	59.01	167.4	1503	1434	4.8	25.47
8.7	F	24.53	126	1229	1004	18.3	50.10
15.7	M	53.62	174.2	1848	1599	15.6	34.46
REE of healthy controls							
Age (years)	Sex	Weight (kg)	Height (cm)	mREE (kcal)	pREE (kcal)	(mREE-pREE) /pREE*100%	mREE/kg BW
4.6	M	19.25	110.1	1388	678	**105**	72.10
0.8	F	9.2	76	738	527	**40**	80.21
4.5	M	17.68	107.9	1125	653	**72.3**	63.63
10.1	M	29	133.4	1256	1106	13.6	43.31
12.5	F	38.85	139.9	1560	1176	**32.6**	40.15

9.7	M	32.33	136.6	1269	896	**41.6**	39.25
18	F	69.14	165.4	1447	1524	-0.05	20.93
15.8	M	38.76	158.8	1313	1326	-0.01	33.88
4.3	F	19.78	107	1180	888	**32.9**	59.66
6.5	F	25.15	124	1177	1009	16.7	46.8
9.6	F	26.36	130.3	1174	1042	12.7	44.54
2.7	F	18.2	99.1	949	963	-0.02	52.14

F female, M male, kg kilograms, cm centimetres, kcal kilocalorie, mREE measured resting energy expenditure, pREE predicted resting energy expenditure, BW body weight, in bold the measurements of REE that over 20% the predicted REE.