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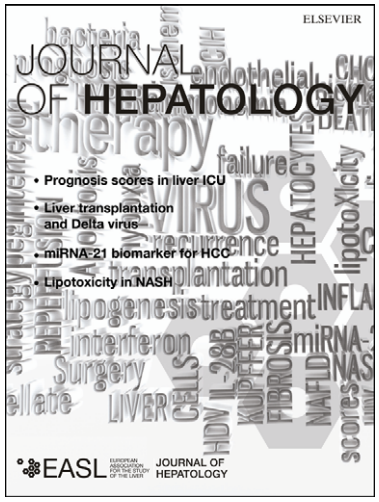
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The Mediterranean Diet Improves Hepatic Steatosis and Insulin Sensitivity in Individuals with Nonalcoholic Fatty Liver Disease

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Abbreviations:

NAFLD: nonalcoholic fatty liver disease

CVD: cardiovascular disease

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MD: mediterranean diet

LF/HCD: low fat high carbohydrate diet

MetSy: Metabolic Syndrome

MRI: magnetic resonance imaging

MRS: magnetic resonance spectroscopy

TG: triglycerides

HDL-C: high density lipoprotein cholesterol

MUFA: mono-unsaturated fatty acids

PUFA: polyunsaturated fatty acids

GINF: glucose infusion rate

HOMA-IR: homeostasis assessment model for insulin resistance

IHL: intrahepatic lipid

Conflict of interest

None the authors of this work have a conflict of interest.

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Background and Aims: Nonalcoholic fatty liver disease (NAFLD) affects up to 30% of the population and signifies increased risk of liver fibrosis and cirrhosis, type 2 diabetes and cardiovascular disease. Therapies are limited. Weight loss is of benefit but is difficult to maintain. We aimed to examine the effect of the Mediterranean Diet (MD), a diet high in mono-unsaturated fatty acids, on steatosis and insulin sensitivity, using gold standard techniques.

Methods: Twelve non-diabetic subjects (6F/6M) with biopsy-proven NAFLD were recruited for a randomised, cross-over 6-week dietary intervention study. All subjects undertook both the MD and a control diet, a Low Fat High Carbohydrate Diet (LF/HCD), in random order with a 6-week washout period between. Insulin sensitivity was determined with a 3-hour hyperinsulinemic-euglycemic clamp study and hepatic steatosis was assessed with localized magnetic resonance ^{1}H spectroscopy (^{1}HMRS).

Results: At baseline, subjects were abdominally obese with elevated fasting concentrations of glucose, insulin, triglycerides, ALT and GGT. Insulin sensitivity at baseline was low (M=2.7±1.0mg/kg/min^{-1}). Mean weight loss was not different between the two diets (p=0.22). There was a significant relative reduction in hepatic steatosis after the MD compared with the LF/HCD: 39±4% versus 7±3% as measured by ^{1}HMRS (p=0.012). Insulin sensitivity improved with the MD, whereas after the LF/HCD there was no change (p=0.03 between diets).

Conclusions**:** Even without weight loss, the MD reduces liver steatosis and improves insulin sensitivity in an insulin resistant population with NAFLD, compared to current dietary advice. This diet should be further investigated in subjects with NAFLD.

KEYWORDS: Liver fat, cardiovascular risk, diet, fatty acids, insulin resistance

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"Nonalcoholic fatty liver disease" (NAFLD) is an umbrella term encompassing simple steatosis, as well as nonalcoholic steatohepatitis (NASH) .NASH can lead to cirrhosis, liver failure and hepatocellular carcinoma in up to 20%; and NASH-associated liver disease is increasing rapidly as an indication for liver transplantation in the United States (1, 2).

Excess hepatic fat deposition is a hallmark of NAFLD. 30% of adults in developed countries are suggested to have excess fat accumulation in the liver (3, 4), and this figure can be as high as 50% amongst individuals with Type 2 diabetes, and 80% in the centrally obese (5, 6). A defect in insulin sensitivity is the key pathogenic feature of type 2 DM and NAFLD. In fact individuals with NAFLD almost invariably have at least one other clinical feature of insulin resistance: elevated serum concentrations of triglycerides (TG), lowered serum concentrations of high density lipoprotein cholesterol (HDL-C), impaired glucose tolerance, central adiposity and hypertension. This constellation of features is termed the Metabolic Syndrome (MetSy) and provides an estimate of cardiovascular disease (CVD) risk, but can also indicate the degree of insulin resistance. Although not formally recognised as part of the MetSy, NAFLD is closely allied with this Syndrome (7).

Studies suggest that the severity of insulin resistance is correlated with the likelihood of progression from benign steatosis to NASH and the development of fibrosis. (5, 6, 8, 9). Therefore therapies that improve insulin sensitivity are also of benefit in NAFLD. Furthermore, as the prevalence of NAFLD, obesity and Type 2 DM are all associated with an increased caloric intake and a sedentary lifestyle (10);. lifestyle modification remains the therapy of choice for NAFLD, particularly given the size of the affected population.

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Studies examining optimal dietary strategies for NAFLD are few. The ideal diet would lead to a reduction of steatosis and an improvement in insulin sensitivity. A previous dietary comparison suggested that the Mediterranean Diet (MD), a diet high in monounsaturated fatty acids (MUFA) was the only one of three diets to reduce serum alanine aminotransferase (ALT) levels (11). A recent meta-analysis demonstrated that omega -3 fatty acids, found in the MD, were beneficial in reducing hepatic steatosis (12).

The MD has been extensively investigated in terms of benefits in relation to reduction of cardiovascular risk (13) and improvement in insulin sensitivity (14-19), however studies specifically examining its effect on NAFLD have not been performed. We hypothesized that in individuals with NAFLD; a MD intervention would improve insulin sensitivity and reduce steatosis to a greater degree than the currently recommended diet.

METHODS

Subjects. The criteria for inclusion in the study were: evidence of steatosis both on ultrasonography and histology (>Grade 1 steatosis [33% hepatocytes steatotic]) (20); the presence of the MetSy, as defined by the presence of at least three clinical features using National Cholesterol Education Program Adult treatment Panel III (NCEP ATP III) criteria (21); as well as consumption of no more than seven/ten standard alcoholic drinks per week for women/men. Subjects with Type 1/2 diabetes were excluded due to concerns regarding the accuracy of low dose hyperinsulinaemic-euglycaemic clamp studies in subjects on insulin or hypoglycaemic agents.

Between May 2009 and April 2011, subjects were recruited from the Liver Clinic and the Cardiovascular Risk Clinic at St Vincent's Hospital, Melbourne. Informed written consent was obtained and all subjects underwent ultrasound-guided liver biopsy.

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Study design. The study was a randomised, cross-over intervention study. We compared the MD group to a comparison diet group, rather than simply to standard care, in order to avoid the placebo effect of undertaking a study diet. The comparison diet chosen was a low fat-high carbohydrate diet (LF/HCD) based both on the Australian National Heart Foundation and American Heart Association dietary recommendations (22, 23). This diet is currently suggested as the optimum diet for patients with cardiovascular risk factors, and therefore is likely to be similar to the dietary advice currently given to Australian patients with NAFLD.

Subjects were randomised to commence either the MD or the LF/HCD, before following onto the second diet after a six-week washout period. Baseline testing on the day of diet commencement involved a fasting blood draw and anthropometry as well as a three hour hyperinsulinaemic-euglycaemic clamp study for the assessment of peripheral insulin sensitivity. During the clamp study, subjects received an hour of dietary instruction from an accredited practising dietitian who provided recipes and a 2-weekly meal plan for the implementation of both the test and control diets.

Following the clamp study, subjects underwent the [1]HMRS. They were then provided with two weeks supply of food, and the diet commenced on that day.

Subjects attended two-weekly appointments with the dietitian during the 6-week diet, where their weight was checked and food diary reviewed for compliance. At the six-week, end-of-study appointment, all baseline testing was repeated.

There was no prescription for activity levels in the study. Subjects recorded their activity over seven days with a pedometer at baseline for each diet, and all patients were advised to maintain their current level of exercise during the two diet periods.

Dietary Interventions. A six week intervention period for both the study and control diets were chosen. Given the high cost of keeping subjects on a whole diet

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intervention, this timeframe was based on previous studies demonstrating effects within this study period (24). Each participant completed a 7-day food diary using household measures, in the week prior commencement and end-of-diet data collections (baseline and end of week 6). The food records were then analysed using FoodWorks 2009 (Version 6.0, Xyris Software, Brisbane, Australia) to determine average daily nutrient intakes and to evaluate compliance with the interventions. This dietary analysis programme utilises the Australian nutrition database NUTTAB 2006 (NUTTAB 2006, Food Standard Australia New Zealand [FSANZ], Canberra, Australia) and Australian Fatty Acid Rev 0.6 (Royal Melbourne Institute of Technology, Melbourne, Australia).

The intervention diet was based on a reconstruction of the traditional Cretan MD, using detailed descriptive food data reported in the Seven Countries Study (25, 26). In addition, traditional recipes and food preparation techniques were sourced from Cretan cook books (26, 27). We have successfully implemented this traditional MD in a randomised controlled trial in the management of Type 2 DM (28) (29).
The majority of foods (70%) on the intervention diet were provided free to subjects: olives, dried fruit, nuts, Greek yoghurt, fish and extra virgin olive oil. To facilitate compliance, subjects were provided with pre-cooked meals. Both diets were prepared and packed into individual servings by a contracted catering service (Zouki P/L, Fitzroy, Australia). Subjects were advised to consume the diet ad libitum, record their consumption in a food diary, and discard the remainder. Subjects were advised that they could drink up to two standard drinks of alcohol per day for five days of the week. The exact diet used is detailed in Appendix 1.

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The MD was high in monounsaturated fats (MUFA) from olive oil; and contained also omega-3 polyunsaturated fatty acids (ω3PUFA), from both plant and marine sources. The approximate macronutrient composition of the diet was 40% energy from fat (MUFA and ω3PUFA), 40% from carbohydrate and 20% from protein. .

The LF/HCD,was based on the both the Australian National Heart Foundation Diet (30), and the American Heart Association Diet (23) and the exact diet used is detailed in Appendix 1. It was low in saturated and unsaturated fat and higher in carbohydrate than the MD. The small amount of fat that was included was predominantly ω6 PUFAs. It was also high in wholegrain carbohydrate foods and had an approximate macronutrient composition of 30% energy from fat, 50% from carbohydrate and 20% from protein. As for the MD, compliance was facilitated by the provision of prepared meals and ingredients. Alcohol intake guidelines for the LF/HCD were the same as for the MD.

Hyperinsulinemic-Euglycemic Clamp Studies. After a 10- to 12- hour overnight fast, subjects were admitted to the Endocrine Testing Centre, for a baseline blood draw for lipid profile, HbA1c, plasma insulin, C-peptide, electrolyte panel, blood cell count and liver enzymes. Intravenous cannulae were inserted into an antecubital vein for blood sampling and the opposite antecubital vein to allow dual infusion of insulin at 40mU/m²/min and 25% dextrose solution in water. Actrapid insulin (Novo-Nordisk, Bagsvaerd, Denmark) was diluted to a concentration of 100mU/mL. Insulin was administered as a continuous infusion at the rate of 40mU/m²/min for 180 minutes as previously described (30). A glucose analyser measured the plasma glucose concentration every 10 minutes after the commencement of the insulin infusion by automated glucose oxidation method (Glucose Analyser 2, Beckman

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Instruments, Fullerton, Calif., USA). A variable infusion of 25% glucose was adjusted based on predictions of required glucose infusion rates using the Oxford Clamp V1.0 computer program. Samples were collected at baseline and from 150-180 minutes in 10 minute intervals for determination of steady state plasma glucose (glucose infusion rate [GINF]), and serum insulin concentrations. Insulin and glucose samples were immediately placed on ice and then centrifuged at 4° C, 3000 rpm for 10 minutes and stored at -80° C for later analysis.

In addition to the clamp studies, which measured peripheral insulin sensitivity, fasting glucose and insulin concentrations were measured at each time point to provide a fasted score which is reflective of hepatic insulin resistance. The homeostatic model assessment for insulin resistance (HOMA-IR) was calculated as per the following equation (31):

Fasting plasma glucose (mmol/L) x fasting plasma insulin (mU/L) / 22.5

Magnetic Resonance Studies. Hepatic steatosis was measured using magnetic resonance spectroscopy (^{1}H MRS) which calculated intrahepatic lipid (IHL), considered the gold standard for quantification of hepatic steatosis

All MRI studies were performed on an Avanto 1.5T system (Siemens, Erlangen Germany). A 3.0x3.0x3.0 cm volume of interest was centred within the right lobe of the liver (avoiding major vessels and ducts) and in the right vastus lateralis muscle belly(32). On a few occasions when body habitus prohibited use of vastus lateralis, the vastus medialis was used for all scans in that individual (4, 33).

All voxel positions were documented and saved on the initial MRI examination to aid reproducibility on subsequent scans. Hepatic and muscle spectra were acquired using the PRESS (point resolved spectroscopy) technique (TR = 3000 ms, TE = 35 ms, 16

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measurements, 1024 sample points).A free breathing technique was used. In vivo IHL concentrations were determined as the percentage of the methylene resonance to water (i.e. CH2/H2O) corrected for T2 effects Hepatic and muscle spectral data were post-processed by magnetic resonance user interface software (jMRUI version 3.0, EU Project) as we have detailed elsewhere (34, 35). ^{1}H-MRS processing was performed by an experimenter blinded to treatment allocation.

Statistical differences between the parameters studied before and after the diets were analysed by paired Student's t test (continuous variables) and the χ^2 test (discontinuous variables). Repeated measures ANOVA was used to determine differences in measured variables following the MD and following the LF/HCD. An interaction term with diet sequence (commencement of the intervention diet first or second) was included to examine any carry-over effects. Analyses were performed on LF/HCD and MD diet periods. Statistical analyses were performed using
the statistical software package SPSS for Windows 20.0 (SPSS Inc., Chicago, IL). $p < 0.05$ was considered significant.

RESULTS

Fourteen subjects were recruited. One subject was ineligible for the study due to insufficient histological steatosis, and a second subject was excluded after intravenous access difficulties at the first clamp study. Therefore a total of 24 diets were completed by 12 subjects. There were no differences in physical activity at the commencement of the two diet periods as recorded by the pedometer for each subject. The order of the diets for each subject was randomised. The baseline characteristics of the six subjects commencing the MD diet first were not significantly different to those

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of the six subjects commencing the LF/HCD first. Following the washout period, there was again no difference in baseline values in the two diet groups [Fig 2].

At baseline the twelve subjects were abdominally obese with a mean waist circumference of 112±8 cm (males) and 96±7 cm (females). They had elevated mean fasting serum concentrations of glucose (104.5±1.1mg/dL), insulin (90±54 pmol/L), triglycerides (318±100 mg/dL), ALT (49 ± 23U/L) and GGT (57 ± 26U/L). Mean insulin sensitivity at baseline was low (M = 2.7±1.0 mg/kg/min^{-1}). The mean IHL at baseline was elevated at 12.2±2.1 % (Table 1).

In addition, at baseline, liver fat content correlated well with baseline measures of insulin sensitivity: IHL % vsHOMA-IR: r=0.6, p=0.006 [Figure 1a].

A comparison of the changes seen with the two diets can be seen in Table 2.

Importantly although there was a small weight loss with both diets (MD: -1.0±0.5 kg vs –LF/HCD: 2.4±0.6 kg), it was not significant and the difference in weight change between the groups was also not significant (p=0.1). There was also no change in waist circumference or BMI between subjects whilst on the two different diets.

Liver outcomes: Following the MD intervention, the twelve subjects demonstrated a significant relative reduction in liver fat content, IHL%. This represents a mean 39±4% relative reduction in liver fat as measured by IHL% over the 6 week period of the diet. In contrast there was only a 7±2% relative decrease in IHL recorded with the LF/HCD (Figure 2a)..

Subjects on the MD demonstrated a significant improvement in insulin sensitivity (Figure 2b). Both the HOMA-IR and the circulating insulin concentration declined significantly (p=0.008 and p=0.003 respectively) (Figure 2c). The Glucose Infusion Rate (GINF) also improved although the result did not reach significance (p=0.09). In

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contrast, there was no significant improvement in insulin sensitivity with the LF/HCD, and the circulating insulin concentrations did not change on this diet.
Cardiovascular disease risk factor outcomes: Systolic blood pressure declined significantly in subjects on the MD. A similar decline was also seen to a lesser degree with the LF/HCD. There was a non-significant fall in serum triglyceride concentration on the MD, which was not seen with the LF/HCD; and the HDL-cholesterol concentrations did not change significantly with either diet.
The serum ALT and GGT concentrations did not change appreciably with either diet.
There were no gender differences in response to the two diets.

DISCUSSION

Although NAFLD is the rapidly increasing in prevalence and is now a significant cause of chronic liver disease (3, 36), there are a lack of therapeutic options that address both the progression of liver fibrosis and the associated insulin resistance. Several medications including Vitamin E have recently been shown to be ineffective in preventing fibrosis(37), and while other pharmaceutical therapies such as thiazoladinediones have had some success in improving liver histology, they have undesirable side effects such as weight gain(38).
Weight loss via lifestyle modification is the recommended focus of therapy but usually difficult to achieve and maintain in the long term for a variety of reasons (39, 40). Therefore alternative strategies are needed to reduce liver fat and improve insulin sensitivity in subjects with NAFLD.
. This study has demonstrated that in just 6 weeks, an olive-oil rich diet, the Mediterranean Diet, can result in a relative reduction in liver fat, even without a change in weight. This decrease in hepatic steatosis was accompanied by an

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improvement in peripheral insulin sensitivity and a reduction in circulating insulin concentrations.

To our knowledge, this the first trial to demonstrate in a randomised, controlled study, a reduction in liver fat and improvement in insulin sensitivity without weight loss in subjects with NAFLD.

Diet, in particular the amount and type of fat consumed, has been linked to the development of insulin resistance, impaired postprandial lipid metabolism and an increased risk of type 2 diabetes(41)

Previous studies have demonstrated that a MUFA-rich diet improves postprandial glucose; decreases oxidised LDL, LDL cholesterol and TG concentrations without a concomitant increase in HDL cholesterol; and improves glyp-1 responses in insulin-resistant subjects (42, 43) (44).

In our study, subjects on the MD experienced an improvement in HOMA-IR, but no significant improvement in peripheral insulin resistance, as measured by GINF. Circulating insulin concentrations also fell. Given that HOMA-IR could be considered a surrogate measure of hepatic insulin resistance, one interpretation of these results is that the reduction in serum insulin concentrations led to a decrease in de novo lipogenesis with a subsequent fall in hepatic steatosis.

Regulations regarding alcohol intake were the same during both diets, and on average subjects were consuming slightly more alcohol prior to commencing the study, however the difference was not significant. The determination of the level of alcohol consumption deemed harmful to the liver is a subject of debate, particularly as there is evidence that individuals with CVD risk factors benefit from regular low level consumption of alcohol (45). We have demonstrated a reduction in steatosis in both diet groups despite ongoing alcohol intake, and also an improvement in insulin

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resistance and systolic blood pressure, both CVD risk factors. Therefore in this small group of patients, a small regular amount of alcohol does not seem to worsen steatosis or CVD risk.

Therefore, in the light of our new findings, we suggest that an increase in intake of MUFAs and also omega-3 PUFAs, particularly as a replacement for saturated fat and as a higher proportion of the diet than carbohydrate, is beneficial for NAFLD patients. The strength of our study lies in its design as a randomised cross-over study so that each subject served as their own control; however it is a small study and therefore the results need to be verified in a larger group. A further strength of the study is our use of gold standard measurement techniques such as ^{1}HMRS and the hyperinsulinaemic euglycaemic clamp to measure hepatic steatosis and insulin sensitivity respectively. Although our analyses did not demonstrate an effect of sequence of the diets on the outcome, it is possible that there was a small non-statistically significant effect, as the mean serum insulin concentration and HOMA-IR and GINF in the LF/HCD group suggested this group were slightly more sensitive than the MD group. This may have been because half of the LF/HCD group had already completed 6 weeks of the MD. This may have affected the ability for these figures to improve further. This needs to be clarified in a larger study.

The lack of change seen in the liver function tests may have been a result of the small sample size or the short duration of the study; however it does demonstrate a lack of sensitivity of these tools as markers of liver inflammation, as has been previously demonstrated (46, 47).

In summary, we have demonstrated a more significant decrease in hepatic steatosis and a greater improvement in insulin sensitivity with just 6 weeks of the MD than can be achieved by the currently recommended LF/HCD. Furthermore, this is an

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inexpensive, non-toxic therapy which results in overall health improvement, reducing the risk of not only chronic liver disease, but also type 2 diabetes and CVD.

Therefore we suggest that this pilot study presents strong evidence that the MD may become the backbone of NAFLD therapy; however larger longer term studies are needed to explore the durability of the benefits of the MD.

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Figure Legend

Figure 1: Correlations between measures of hepatic steatosis and insulin sensitivity.
Pearson correlations: 1a: IHL% vs HOMA-IR ($p<0.006$); 1b: IHL% vs GINF ($p=0.2$)

Figure 2: Change in hepatic steatosis and insulin sensitivity: between diet comparisons
2a: A significantly greater decrease in intrahepatic lipid % was seen with the MD than the LF/HCD ($p=0.03$).
2b: A significantly greater increase in the glucose infusion rate (GINF) was seen with the MD than with the LF/HCD ($p=0.03$)
2c: A greater fall in serum insulin concentrations was seen with the MD than the LFHCD ($p=0.008$)

Figure 3: Change in intrahepatic lipid for individual subjects across the two diet periods and washout period.

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TABLE 1: Baseline Characteristics (N=12)

	Mean ± SD	Normal range
Age (yrs)	55 ± 14	NA
Gender (M/F)	6/6	NA
BMI (kg/m^2)	32.0 ± 4.2	20.0 – 25.0
Waist Circumference (cm)	112 ± 8 (M) 96 ± 7 (F)	M <102, F <88
Systolic BP (mmHg)	138 ± 20	<130
Diastolic BP (mmHg)	76 ± 9	<80
ALT (U/L)	49 ± 23	< 30
γGT (U/L)	57 ± 26	< 45
Glucose (mg/dL)*	121 (22)	< 111
Insulin (mU/L)*	13.0 (7.8)	< 8.6
HOMA-IR*	4.3 (3.1)	<2.0
Triglycerides (mg/dL)	318 ± 100	<150
HDL-cholesterol (mg/dL)	40 ± 12 (M) 46 ± 11 (F)	M > 40 F > 50
GINF (mg/kg/min-^{1})	2.4 ± 0.3	<7.0**
HFF% (MRI)	17.7 ± 2.7	<5% #
IHL% (^{1}HMRS)	11.2 ± 2.1	<5% #

* Non-normally distributed data are expressed as median ± inter-quartile range

** DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979;237:E214-223

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TABLE 2: Changes seen with MD and LF/HCD

	MD		LF/HCD	
	Baseline	End of diet	Baseline	End of diet
Energy (MJ)/day	8.7 ± 1.9	8.8 ± 1.0	9.0 ± 3.9	7.4 ± 0.1
Protein (%E)	21.4 ± 3.2	**15.8 ± 0.9****	21.0 ± 3.4	**23.5 ± 0.2****
Carbohydrate (%E)	38.4 ± 3.3	33.6 ± 2.4	37.2 ± 5.9	**48.9 ± 2.9****
Fat (%E)	32.2 ± 2.8	**44.3 ± 2.2****	35.7 ± 6.0	**20.7 ± 1.6 ****
Alcohol (g/day) (F)	1.8 ± 2.4	2.5 ± 3.0	4.5 ± 9.0	4.0 ± 4.5
Alcohol (g/day) (M)	3.3 ± 3.9	3.2 ± 5.4	3.2 ± 2.7	2.5 ± 4.7
Fibre (g/day)	27.6 ± 6.7	**36.4 ± 3.1****	26.6 ± 8.4	29.4 ± 4.9
Fatty Acid Ratios: Saturated (%)	41.5 ± 3.3	**30.7 ± 1.5****	38.7 ± 4.0	36.4 ± 3.2
Monounsaturated (%)	41.3 ± 2.9	**51.0 ± 1.4****	43.2 ± 1.6	39.0 ± 3.6
Polyunsaturated (%)	16.8 ± 2.8	18.5 ± 0.8	17.9 ± 3.1	24.4 ± 4.2
BMI (kg/m^2)	31.5 ± 3.9	31.2 ± 3.6	31.5 ± 3.5	30.8 ± 3.9
Weight (kg)	88.3 ± 11.3	87.3 ± 10.3	90.7 ± 13.9	88.3 ± 11.4
WC (cm)	104.8 ± 9.0	102.7 ± 6.0	103.7 ± 8.6	102.2 ± 8.2
Systolic BP (mmHg)	140 ± 21	**127 ± 13****	130 ± 15	**125 ± 14***
Diastolic BP (mmHg)	80 ± 8	77 ± 7	71 ± 8	76 ± 11
Insulin (mU/L)	18.4 (8.6)	**11.7 (5.5)****	13.5 (7.5)	13.8 (5.3)
Glucose (mg/dL)	108 (16)	103 (12)	112 (14)	106 (16)
ALT (U/L)	46 ± 17	42 ± 12	49 ± 28	45 ± 33
γGT (U/L)	52 ± 24	48 ± 32	57 ± 24	54 ± 29
TG (mg/dL)	224 ± 109	201 ± 86	222 ± 133	221 ± 101
HDL-C (mg/dL)	44.5 ± 7.0	42.5 ± 7.7	41.7 ± 9.4	41.9 ± 8.3
HOMA-IR	4.7 ± 1.6	**3.0 ± 1.4****	4.1 ± 2.1	3.9 ± 1.4
GINF ($mg/kg/min^{-1}$)	2.2 ± 0.8	4.2 ± 2.8	2.9 ± 1.9	3.4 ± 1.6
Mean SS Glucose (mg/dL)	110 ± 15	99.4 ± 12	113 ± 16	101 ± 17
Mean SS Insulin (mU/L)	33.0 ± 14.0	28.5 ± 9.1	27.1 ± 8.5	25.2 ± 9.8

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M/I (mg/kg/min^{-1}/mU/L^{-1})	0.067 ± 0.057	0.147 ± 0.308	0.107 ± 0.224	0.135 ± 0.163
HFF %	17.7 ± 12.4	**12.4 ± 10.8****	16.4 ±12.6	13.7 ± 9.0
IHL %	14.2 ± 11.7	**8.6 ± 7.0***	11.2 ± 4.4	10.0 ± 3.6

$p < 0.05$; ** $p < 0.01$,Wilcoxin signed rank test
M/I: ratio of glucose infusion rate (GINF) to mean steady state insulin

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Figure 1a

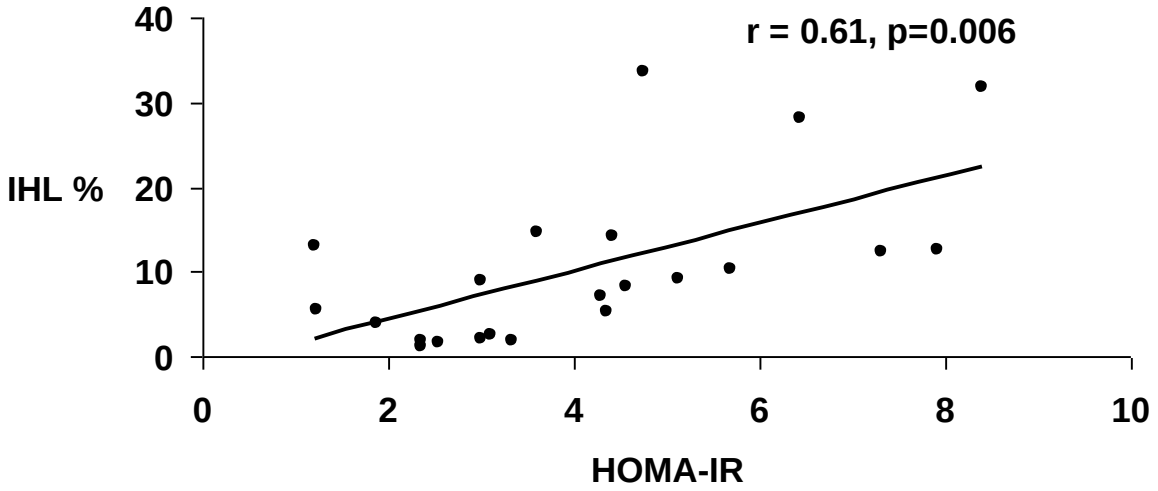


Figure 1b

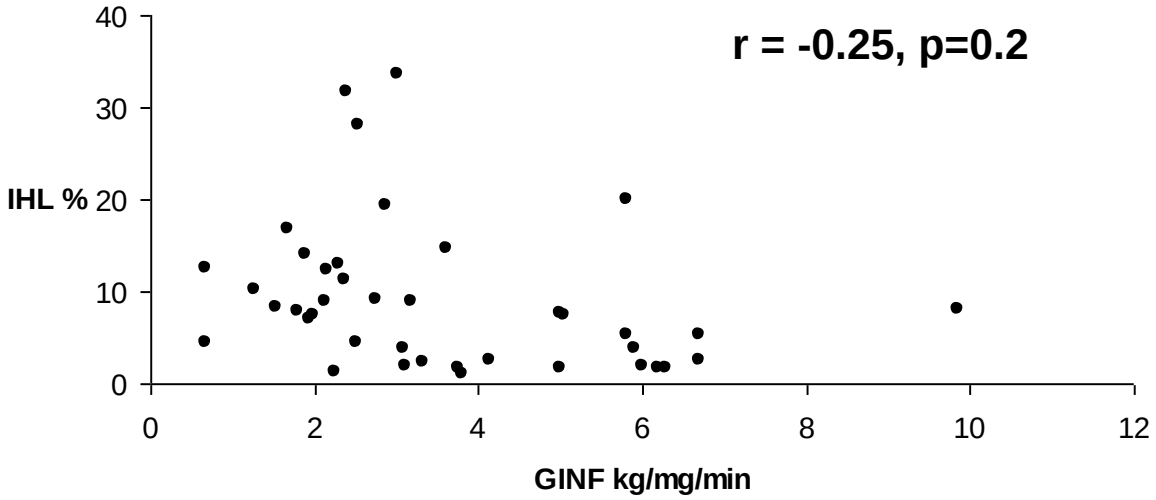


Figure 2a

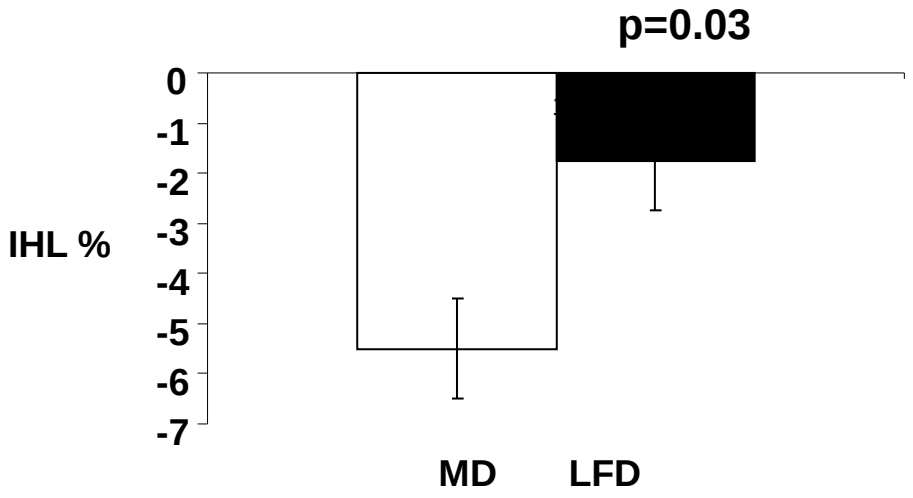


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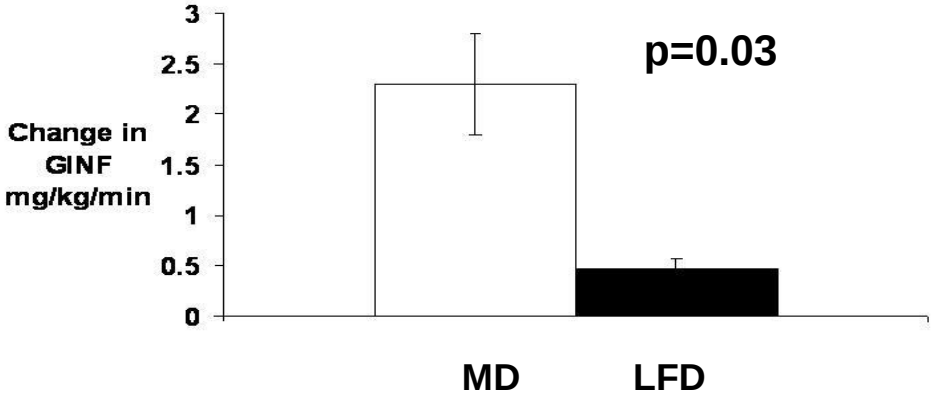


Figure 2c

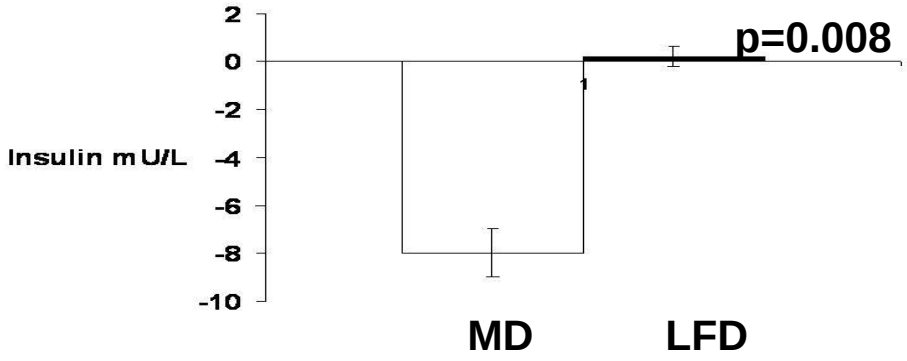
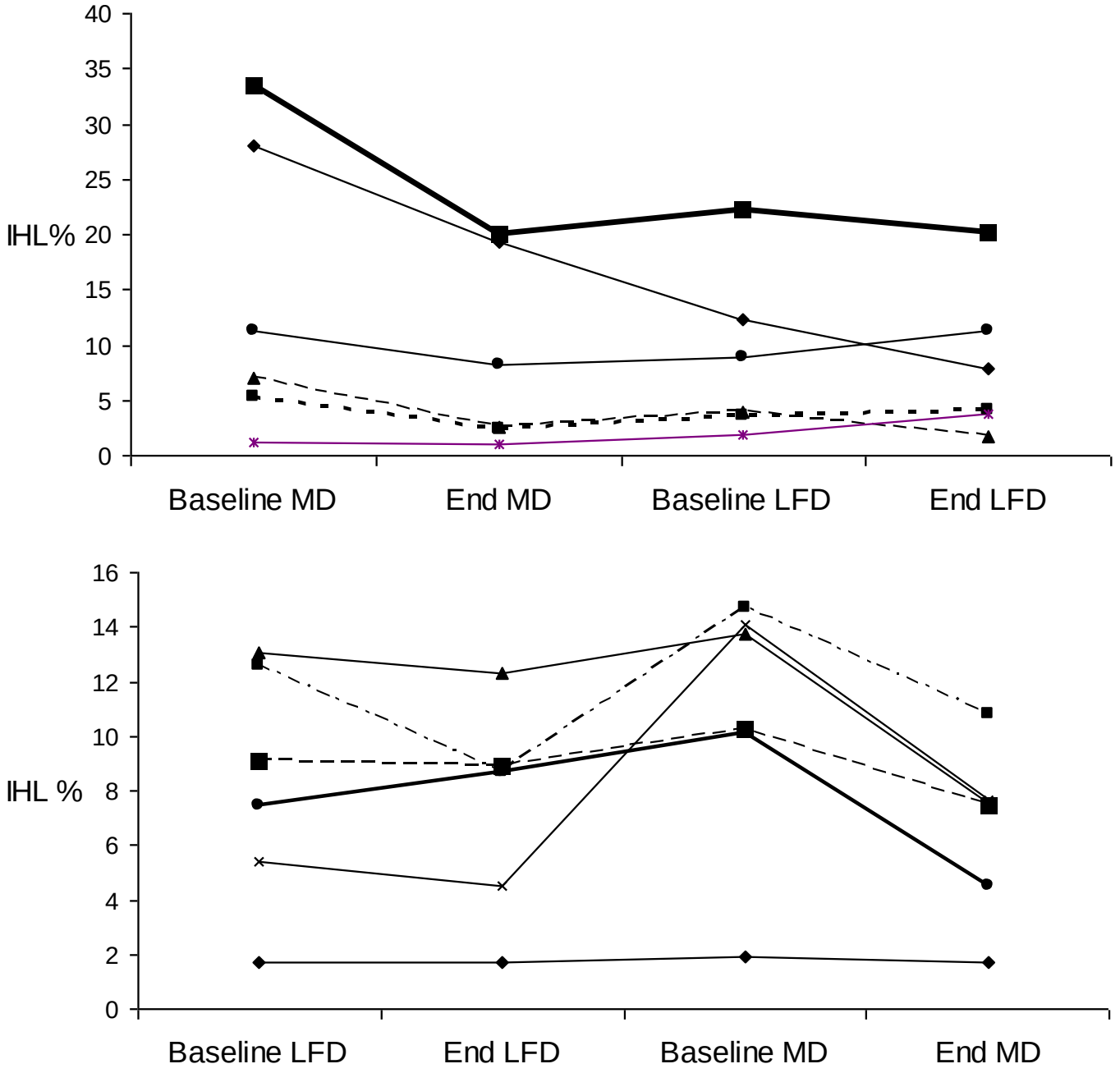


Figure 3


IHL%
40
35
30
25
20
15
10
5
0
Baseline MD
End MD
Baseline LFD
End LFD
IHL %
16
14
12
10
8
6
4
2
0
Baseline LFD
End LFD
Baseline MD
End MD