Project Number:
184

Fasting *de novo* lipogenesis and gluconeogenesis: an investigation into association with markers of insulin resistance in humans

Research Project Report

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Abstract

Background

Insulin resistance (IR), which is typically characterised by hyperinsulinemia, is associated with many metabolic diseases, including Type-2 Diabetes Mellitus, Non-alcoholic Fatty Liver Disease and Cardiovascular Disease. Evidence from rodent models suggests hepatic *de novo* lipogenesis (DNL) and gluconeogenesis (GNG) are upregulated with IR leading to hypertriglyceridemia and hyperglycaemia. However, due to the paucity of human studies investigating both hepatic DNL and GNG, it remains unclear whether these processes are upregulated with IR in humans. Therefore, the aim of this project was to investigate the association of fasting hepatic DNL and GNG with markers of insulin resistance in humans.

Methods

Plasma samples from 157 healthy (free from any known disease) adults who had taken part in previous studies and consumed heavy water for the measurement of hepatic DNL, were utilised. Fasting hepatic GNG was assessed using gas chromatography-mass spectrometry. Participant phenotype and fasting DNL were collated and hepatic DNL and GNG were then compared to anthropometric and biochemical markers of IR.

Results

There were no associations between fasting hepatic GNG and markers of insulin resistance, including fasting plasma insulin ($p=0.51$) and glucose ($p=0.31$), or HOMA-IR ($p=0.68$). Nor was there a correlation between fasting hepatic DNL and GNG ($p=0.49$). However, a positive association between hepatic DNL and both fasting insulin ($r= 0.24$, $p=0.003$) and HOMA-IR ($r= 0.22$, $p=0.006$), was observed. There was notable sexual dimorphism in hepatic GNG with male participants having higher fasting GNG than females ($p=0.002$); hepatic GNG decreased over age in men but not women. Low-density lipoprotein was a very strong predictor of hepatic GNG in men whereas waist circumference was the strongest predictor in women.

Conclusion

These results suggest that in humans, fasting hepatic DNL is upregulated in IR but GNG is not; this is in contrast to the evidence from rodent models. These metabolic processes are poorly understood in humans and further work is needed to characterise inter- and intra-personal variability in DNL and GNG which may give further insight into their roles in IR as well as providing opportunities for therapeutic intervention.

Introduction

In 2016 the World Health Organisation estimated 1.9 billion adults worldwide were overweight, with 650 million of these being obese[1], and this figure is known to be rapidly increasing[1,2]. Obesity is a known risk factor for insulin resistance (IR) which is associated with increased risk of metabolic diseases including Type-2 Diabetes Mellitus (T2DM), Non-alcoholic Fatty Liver Disease (NALFD) and Cardiovascular Disease (CVD)[3–6].

Hyperinsulinemia is an established hallmark of IR[7–9] and individuals may also present with hyperglycaemia and/or hypertriglyceridemia[3,10,11]. It is suggested that increased hepatic gluconeogenesis (GNG) and/or *de novo* lipogenesis (DNL) contribute to elevated plasma glucose and/or triacylglycerol (TAG) concentrations[12]. In humans these two metabolic processes occur almost exclusively in the liver and are regulated by insulin. Briefly, when insulin binds to its receptor on hepatocytes, it upregulates DNL through the sterol regulatory element-binding protein 1c (SREBP-1c) pathway and suppresses GNG through the Forkhead box protein O1 (FOXO1) pathway[13]. Goldstein and Brown hypothesised that within the liver there may be selective IR which develops downstream of the divergence of these two metabolic pathways such that DNL maintains its sensitivity to insulin but GNG becomes resistance[12]. This hypothesis has been supported by rodent models[14,15] but hepatic DNL and GNG are rarely measured in human studies, thus it remains unclear if these processes are upregulated in humans. Recently, Samuel and Shulman suggested instead that IR in peripheral tissues (i.e. adipose tissue, skeletal muscle) causes a systemic increase in the precursors for hepatic DNL and GNG, leading to an increase in these two processes without the liver becoming insulin resistant[16]; this too has yet to be demonstrated *in vivo* in humans.

Therefore, the aim of this project was to characterise hepatic DNL and GNG in healthy adults, across a wide range of insulin sensitivities after an overnight fast using established markers of IR such as fasting plasma insulin and glucose concentrations or HOMA-IR. Furthermore, the relationship of DNL and GNG with factors which are associated with IR[17–21] (e.g. intrahepatic triglyceride (IHTG) content, body mass index (BMI), waist circumference, triacylglycerol (TAG), non-esterified fatty acids (NEFA) and cholesterol) will be explored. As it is important to understand the effects of dietary and pharmacological intervention on hepatic DNL and GNG two such interventional studies will also be explored here.

Materials and Methods

Participants and collation of studies

I was responsible for the measurement of GNG and the compilation of the subsequent database and analysis. Data from 157 participants was collated from previous and on-going studies[22–26] (known as LFCCS (23 participants), FOS (22 participants), BHF (60 participants), Sugar Study (16 participants), SOS (16 participants) and SMASH (20 participants)). In all studies apart from LFCCS, BHF, and Sugar Study, participants had two separate study days to assess the effects of a pharmacological or dietary intervention. In such cases only data from their first (baseline) visit was included in the analysis. The effects of any intervention study were analysed separately.

For all studies, healthy participants were recruited through the Oxford BioBank (OBB)[27] or from the wider Oxfordshire area by advertisement. All volunteers were non-smokers, free from any known disease, not taking medication known to affect glucose or lipid metabolism and consumed alcohol within the recommended limits. All subjects provided informed written consent, and studies were approved by the relevant Research Ethics Committees.

In all studies, participants were asked to avoid alcohol and strenuous exercise for 24 hours prior to their study day. The evening prior to the study day, subjects consumed deuterated water (^2H_2O) (3g/kg body water) for the measurement of fasting hepatic GNG and DNL. Participants came into the clinical research unit the next morning after an overnight fast and a cannula was inserted into an antecubital vein on one arm from which a fasting blood sample was taken.

Analysis of body composition and liver fat

Body composition and body fat percentage were measured using dual-energy X-ray absorptiometry (DEXA) and IHTG was measured using magnetic resonance spectroscopy (^{1}H-MRS), as described[28].

Analytical methods

Whole blood was collected into heparinised syringes (Sarstedt, Leicester, UK) and centrifuged 4°C to isolate plasma. Concentrations of glucose, NEFA, total and high-density lipoprotein (HDL) cholesterol and TAG were analysed on a semi-automatic analyser (ILab 600/650 clinical chemistry; Werfen, Warrington, U.K.) and plasma insulin levels were determined by radioimmunoassay as previously described[29]. Very low-density lipoprotein (VLDL) fractions (S_f 20–400) were isolated using density gradient ultracentrifugation and this fraction was then separated by immunoaffinity chromatography[29] for measurement of hepatic DNL.

Extraction of Glucose

Plasma samples were deproteinised with 500ul of ethanol and centrifuged for 5mins at 2500rpm. The supernatant was collected and dried in a centrifugal evaporator for 45mins. 100ul of methylhydroxamine hydrochloride in pyridine (2%w/v) was added and samples were capped and heated to 90°C for 2hrs. Once cooled, 50ul of BSTFA+1%TCMS was added to react with the samples at 120°C for a further 15mins. Samples were cooled and dried in the centrifugal evaporator and then reconstituted in 500ul of decane. Tracer enrichment in plasma glucose was analysed by gas chromatography-mass spectrometry (GC-MS) with selected ion monitoring for ions with a mass-to-charge ratio (m/z) of 319 (M+0) and 320 (M+1).

Analysis of GNG

GNG was measured as fractional gluconeogenesis using the "average" method first described by Chacko et al[30]. Briefly, at low plasma water enrichments, it is assumed that a single hydrogen covalently bound to any of the carbons on a glucose molecule will be substituted for a deuterium during gluconeogenesis. It is therefore possible to calculate the average deuterium enrichment of the glucose derivative by analysing the M+1/M+0 ratio (m/z of 320/319)[31], converting into mole percent enrichment and dividing by the number of possible hydrogen labelling sites (in our case 5)[30,32,33]. Fractional GNG was calculated by diving the average deuterium enrichment of glucose by plasma water $^{2}H_2O$ enrichment (measured via IRMS analysis using Finnigan GasBench-II; ThermoFisher Scientific, Loughborough, UK). This was converted into a percentage representing the proportion of total glucose in the blood that came from GNG.

Glycogenolysis, the only other form of endogenous glucose production, will only label the hydrogen on carbon 2 of the glucose molecule[32,34]. Therefore, as our glucose derivative only included carbons 3, 4, 5 and 6[35,36], we can be certain that any labelling must have occurred during GNG.

Analysis of DNL

Hepatic DNL was assessed by determining the incorporation of deuterium from $^{2}H_2O$ into VLDL-TAG palmitate using GC-MS. The monitoring ions had m/z of 270 (M+0) and 271 (M+1) and percentage DNL calculated as described[9].

Calculations and Statistical Analysis

The homeostatic model assessment for insulin resistance (HOMA-IR) was calculated based on fasting glucose and insulin measurements as previously described[37] and was used as a marker of insulin resistance[38]. The serum concentration of low-density lipoprotein (LDL) was calculated using the LDL Friedewald equation[39].

All data were analysed using SPSS for Mac. V.27 (SPSS, Chertsey, UK) and initially tested for normality using the Shapiro-Wilk test. To determine associations between two variables, Spearman's Rank correlations were done in the first instance followed by t-testing (with the independent variable split by the median) or the Mann Whitney U test for non-parametric data. T-tests or Mann Whitney U tests were also used to compare the difference in a variable between two groups. To determine the effect of interventions the related-samples Wilcoxon Signed Rank test was used due to the small sample sizes. Multiple linear regression was run using the forward method to establish the best predictors for DNL and GNG. Independent variables were entered only if they exhibited a significant correlation and were then individually checked for linearity with the dependant variable before being entered. Homoscedasticity was checked using scatterplot of standardised residual over standardised predictors and the residuals were checked for approximately normal distribution using a normal P-P plot. Statistical significance was set at $p< 0.05$.

Results

Participant Characteristics

The characteristics of the 157 participants are presented in *Table 1.* Participants were aged between 27 – 64 years, predominantly female (61%), overweight (BMI between 19.5 – 42.3 kg/m^2) and HOMA-IR between 0.3 – 23.5.

Table 1. Participant characteristics

Participants (Males/Females)	157 (61/96)
Age (years)	47 (27 - 64)
BMI (kg/m^2)	27.1 (19.5 - 42.3)
Waist Circumference (cm)	90 (69 - 186)
Intrahepatic triglyceride (%)	1.9 (0.1 – 36.4)
HOMA-IR	2.5 (0.3 – 23.5)
Fasting plasma biochemistry	
Glucose (mmol/L)	5.3 ± 0.1
Insulin (mU/L)	12.9 ± 0.6
TAG (µmol/L)	1264 ± 68
DNL (%)	5.8 (0.08 – 35.5)
GNG (%)	27.4 (5.2 – 75.7)

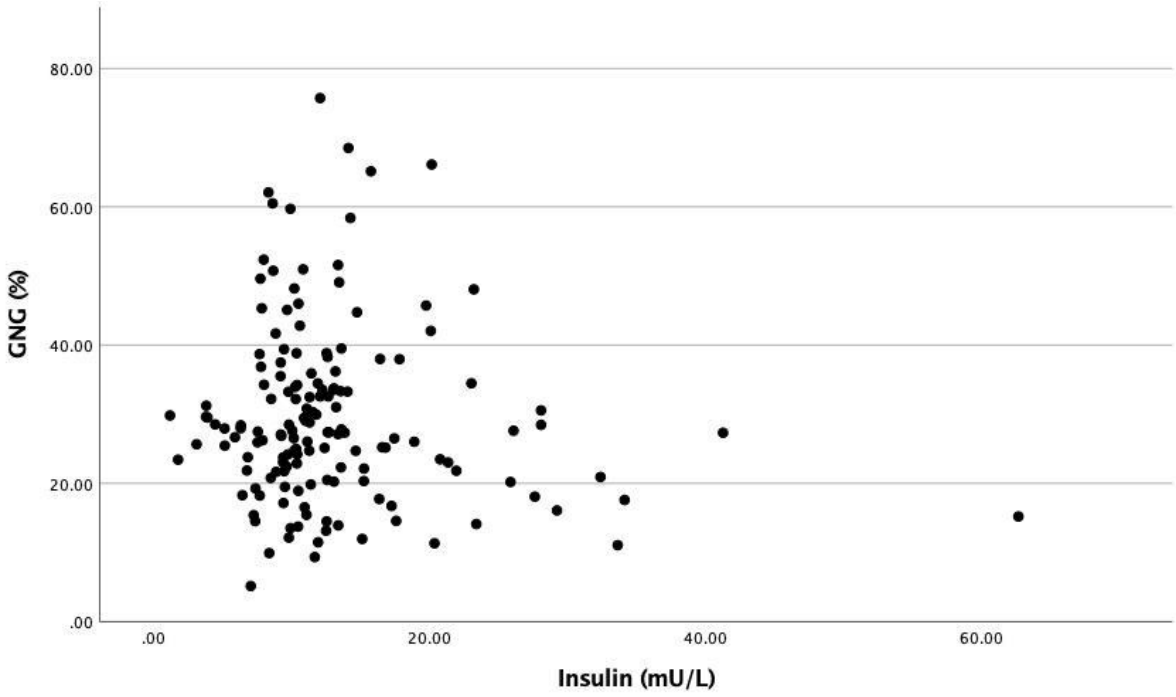
Data are presented as median (min - max) or mean ± SEM.
Abbreviations: BMI, Body Mass Index; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; TAG, triacylglycerol; DNL, *de novo* lipogenesis; GNG, gluconeogenesis.

The association of fasting DNL and GNG with fasting insulin and glucose

There was no correlation between DNL and GNG across the entire group ($r= 0.06$, $p=0.49$). Similarly, no association was found between GNG and fasting insulin in the entire cohort (Fig. *1A*) or when split by sex (*data not shown*), nor was there any association with HOMA-IR. In line with previous

observations, we found DNL had a significant positive correlation with insulin (r= 0.24, p=0.003) (Fig. *1B*) and HOMA-IR (r= 0.22, p=0.006). We found no significant relationship between fasting glucose levels and either DNL or GNG in our cohort (Supplementary Fig. *1*).

A



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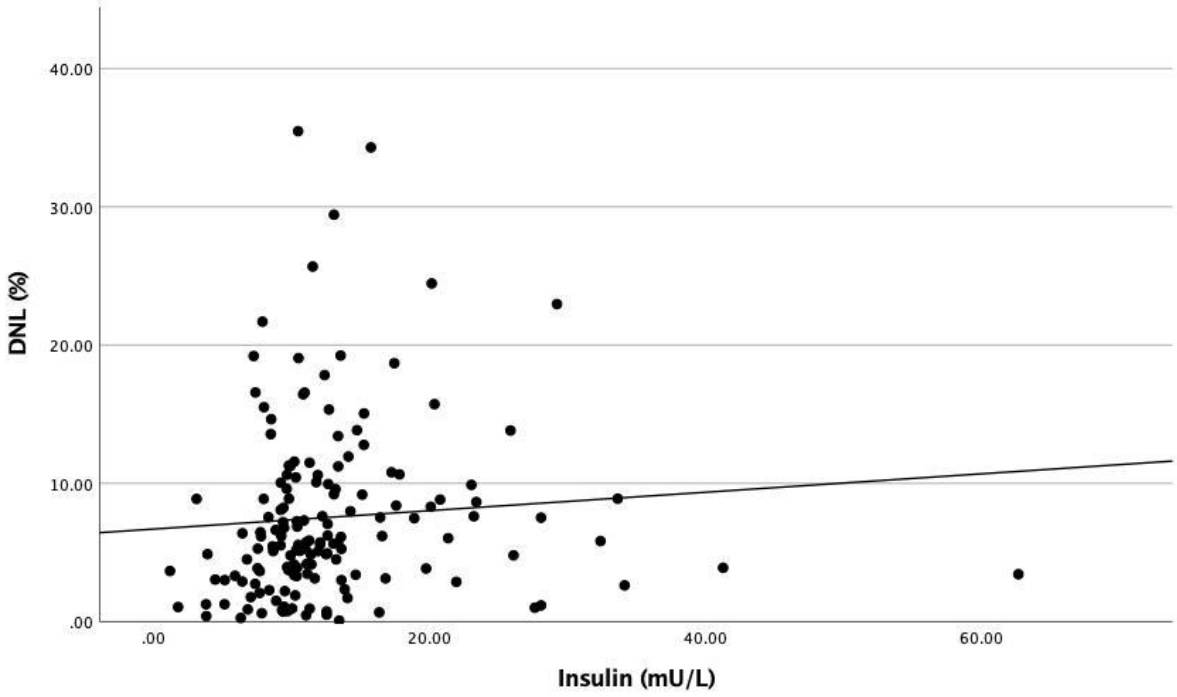

Figure 1. Association between fasting plasma insulin and (A) GNG and (B) DNL in men and women.
Data is presented with line of best fit for DNL where a significant relationship is observed.

To explore the relationship between IR and hepatic DNL and GNG, an "IR subgroup" was identified. This subgroup had both fasting glucose and fasting insulin levels above the median and a HOMA-IR above 2.5, the latter based on previous work to define the prevalence of IR[40–42], and was compared to the rest of the cohort (known as "controls"). Compared to the controls, the IR cohort had significantly higher BMI, waist circumference, total fat percentage, visceral fat, IHTG, plasma TAG and VLDL–TAG concentrations (*Table 2*). However, there was no difference in fasting GNG between those defined as IR and controls and although DNL was higher in the IR compared to the control group this was not significant (p=0.084) (*Table 2*). Although there were more men in the IR cohort than women, splitting by sex also showed no differences in DNL and GNG between the two groups.

Table 2. Characteristics of controls and Insulin Resistant individuals

	Controls (n=104)	Insulin Resistant (n=53)
Age (years)	47	47
Sex (Male/Female)	32/72	29/24**
BMI (kg/m^2)	25.9 (19.5 - 35.2)	28.9 (22.2 - 42.3)**
Waist Circumference (cm)	86 (69 - 115)	97 (74 - 186)**
Total fat (%)	34.2 (15.8 - 93.3)	37.4 (23.6 - 51.2)
Visceral fat (g)	654.8 (0.2 - 2531)	1560.3 (304 - 3720.8)**
IHTG (%)	1.6 (0.3 - 24.4)	6.7 (0.1 - 36.4)**
Fasting plasma biochemistry		
TAG (µmol/L)	1021.8 ± 62.8	1737.8 ± 136.8**
NEFA (µmol/L)	522.1 ± 19.7	537.4 ± 25.6
Total cholesterol (mmol/L)	5.2 ± 0.1	5.1 ± 0.1
HDL cholesterol (mmol/L)	1.5 ± 0.04	1.2 ± 0.04**
Non-HDL cholesterol (mmol/L)	3.7 ± 0.1	3.9 ± 0.1
LDL cholesterol (mmol/L)	3.3 ± 0.09	3.1 ± 0.1
VLDL-TAG (µmol/L)	473.8 ± 47	870.0 ± 74.3**
DNL (%)	**5.4 (0.08 – 35.5)**	**7.5 (0.7 – 34 3)**
GNG (%)	**27.4 (5.2 – 68.5)**	**27.6 (11.1 – 74.7)**

Data is presented as median (min – max) or mean ± SEM. ** p< 0.01
Abbreviations: BMI, Body Mass Index; IHTG, intrahepatic triglyceride; TAG, triacylglycerol; NEFA, non-esterified fatty acids; HDL, high-density lipoprotein; LDL, Low-density lipoprotein; VLDL, very low-density lipoprotein; DNL, *de novo* lipogenesis; GNG, gluconeogenesis.

The influence of sex and age on DNL and GNG

We assessed the effect of sex on hepatic GNG and found males had significantly higher fasting GNG than females (p=0.002, Fig. *2A*). In contrast, we did not observe any sexual dimorphism in fasting hepatic DNL (p=0.46, Fig. *2B*). In a multiple linear regression analysis, sex was found to be the only significant predictor (p=0.01) of fasting hepatic GNG.

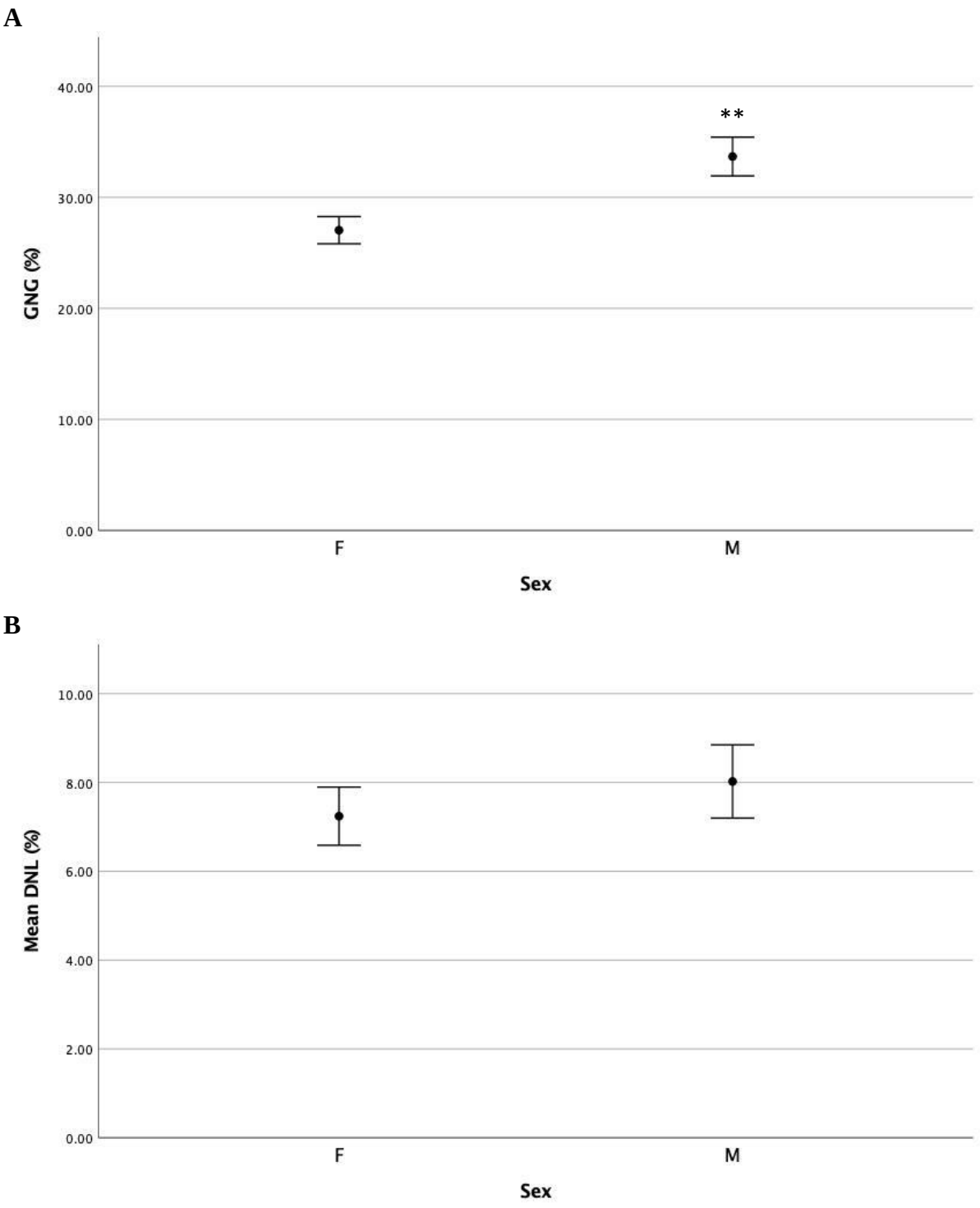

Figure 2. The effect of sex on fasting (A) GNG and (B) DNL.
Data are presented as means ± SEM. ** p< 0.01
Abbreviations: GNG, gluconeogenesis; DNL, *de novo* lipogenesis; F, female; M, male.

We investigated the influence of age on fasting hepatic DNL and GNG and found a trend (p=0.06) for an inverse association (r= -0.15) between age and hepatic DNL across the whole group (Fig. *3*); this was more prominent in women (r= -0.17, p=0.09) than men (r= -0.04, p=0.778) (*data not shown*). In contrast, the association between age and GNG showed a significant negative relationship in men (r= -0.28, p=0.03) which was not seen in females (r= -0.02, p=0.89) (Fig. *4A and B)*. Multiple linear

regression models showed age was a significant predictor of GNG in men (beta coefficient= -0.732, p=0.002) but not women (p=0.841).

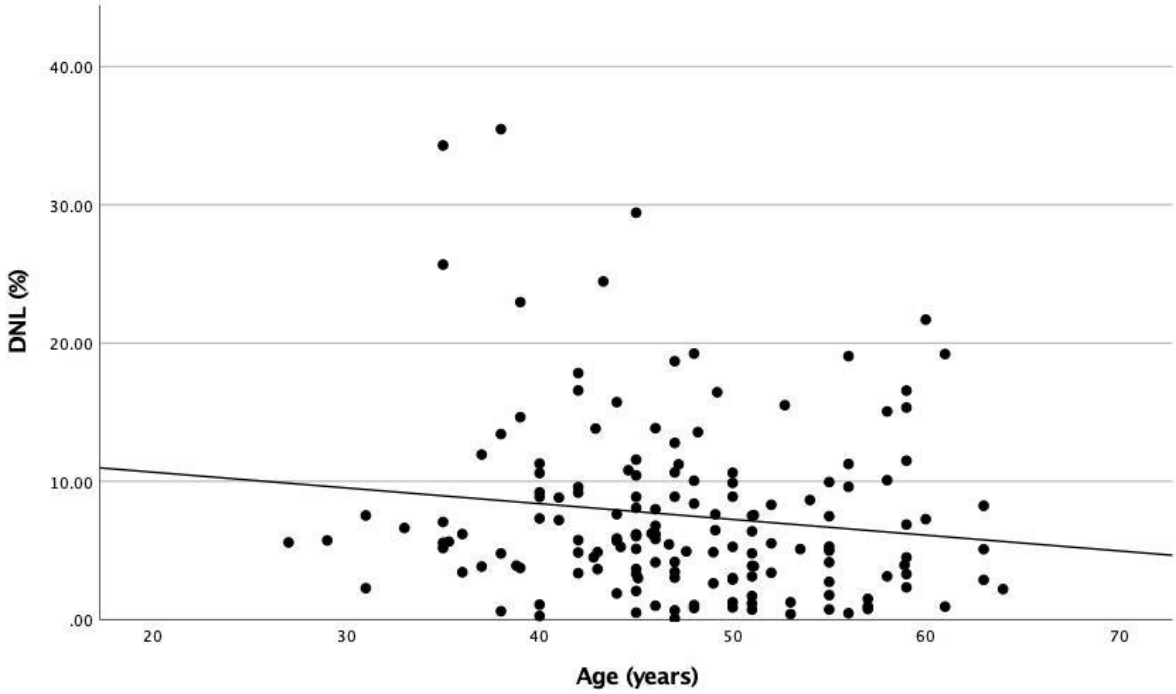

Figure 3. The influence of age on fasting hepatic DNL in men and women.
Data is presented with a line of best fit.

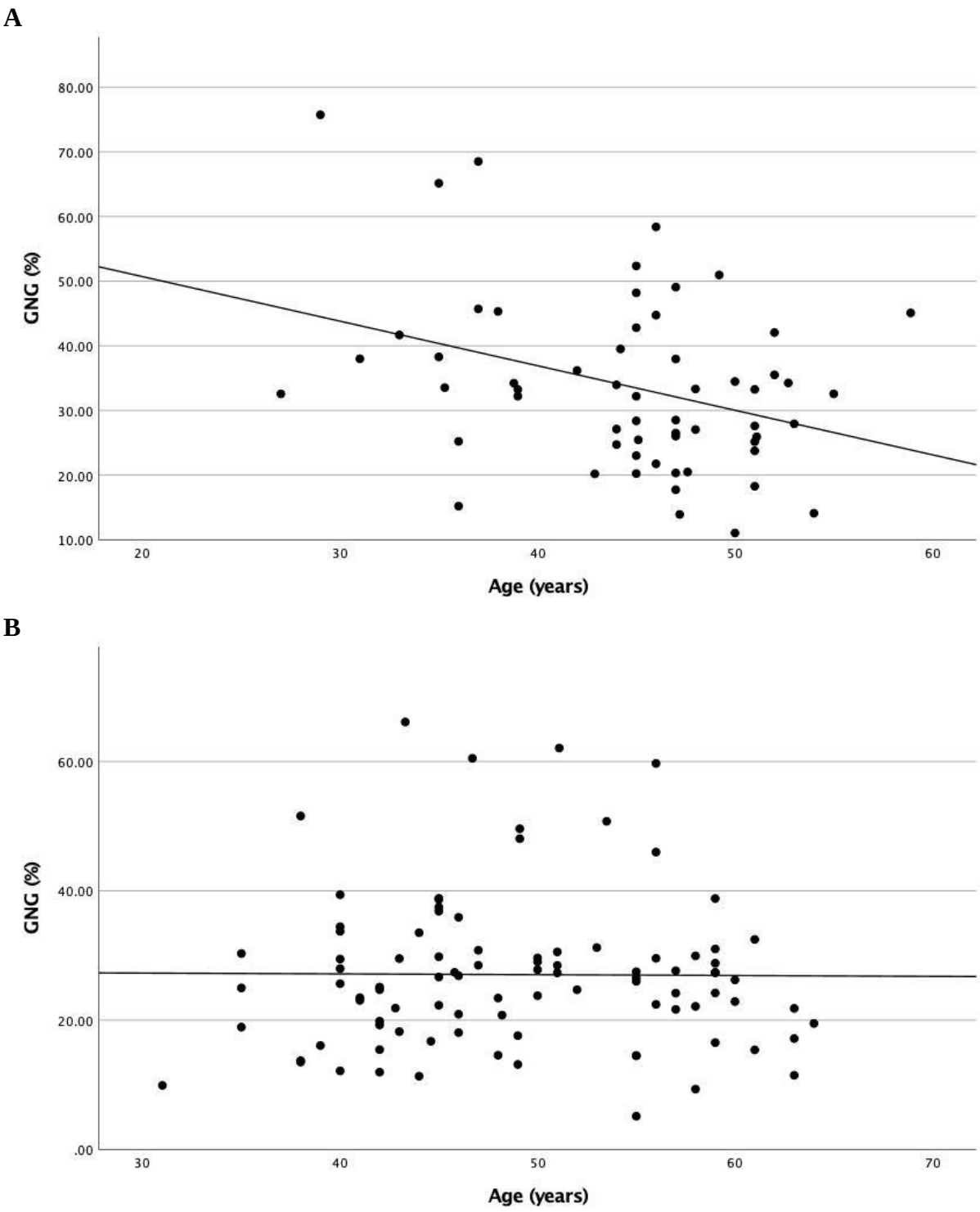

Figure 4. The influence of age on fasting hepatic GNG in (A) men and (B) women.
Data is presented with a line of best fit.

Associations of fasting DNL and GNG with anthropometric measurements

As body fatness is often associated with IR[17,20], we investigated the association between BMI, waist circumference and body composition with hepatic DNL and GNG (*Table 3*). No relationship was found between hepatic DNL and BMI across the group, however there was a strong association between DNL and both waist circumference and total body fat in women which was not seen in men (*Table 3*).

In contrast, GNG was inversely associated with BMI in men only and, although BMI did not correlate, GNG was positively associated with waist circumference, total fat and inversely associated with lean mass in women (*Table 3*). Multiple regression analysis in women showed that waist circumference was the strongest predictor of GNG from variables measured with a beta-coefficient of 0.36 (p=0.01). Neither visceral fat nor IHTG content had a significant influence on DNL or GNG in either sex, although there was a trend (p=0.07) for a positive correlation between visceral fat and GNG in women.

Table 3. Correlations between anthropometric data and DNL or GNG

	DNL		GNG	
	Male	Female	Male	Female
BMI	-0.04	0.13	-0.27*	0.13
Waist Circumference	0.01	0.29**	-0.09	0.23*
Total fat	0.01	0.23*	-0.05	0.24*
Lean mass	-0.01	-0.23*	0.05	-0.24*
Visceral fat	0.11	0.23	-0.05	0.28
IHTG	0.15	0.06	-0.07	0.07

Data is expressed as r values. * p<0.05, ** p<0.01
Abbreviations: DNL, *de* novo lipogenesis; GNG, gluconeogenesis; BMI, Body Mass Index; IHTG, intrahepatic triglyceride.

The association of fasting DNL and GNG with plasma fatty acids and lipids

Changes in plasma fatty acids and lipids have been associated with IR[19,21] so their relationship with DNL and GNG was investigated (*Table 4*). DNL showed an inverse association with NEFA as has been previously reported[43] which was substantially stronger in men than women. Positive associations between DNL and TAG, non-HDL cholesterol and VLDL-TAG were also observed in both sexes.

Table 4. Correlations between fasting DNL or GNG and plasma fatty acids and lipids

	DNL		GNG	
	Male	Female	Male	Female
NEFA	-0.32*	-0.12	0.08	0.14
TAG	0.46**	0.30**	0.11	-0.00
Total cholesterol	0.22	0.17	0.37**	-0.08
HDL cholesterol	-0.15	-0.15	-0.08	-0.08
Non-HDL cholesterol	0.28*	0.22*	0.39**	-0.07
LDL cholesterol	0.10	0.16	0.40**	-0.04
VLDL	0.39**	0.27**	0.15	-0.01

Data are presented as r values. * p<0.05, ** p<0.01
Abbreviations: DNL, *de* novo lipogenesis; GNG, gluconeogenesis; NEFA, non-esterified fatty acids; TAG, triacylglycerol; HDL, high- density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

GNG was very strongly positively associated with LDL cholesterol in men (Fig. *5A*) but not in women (Fig. *5B*) and similar strong relationships were seen with total cholesterol and non-HDL cholesterol. Multiple linear regression showed that LDL cholesterol and age were the only significant predictors of GNG in men (beta coefficients of 6.18 and -0.74 respectively, p=0.002 for both) with an R^2 value of

0.26 for the model as a whole. No associations were observed between GNG and plasma fatty acids or lipids in women. No significant correlation was found between DNL and GNG in either men (p=0.50) or women (p=0.89).

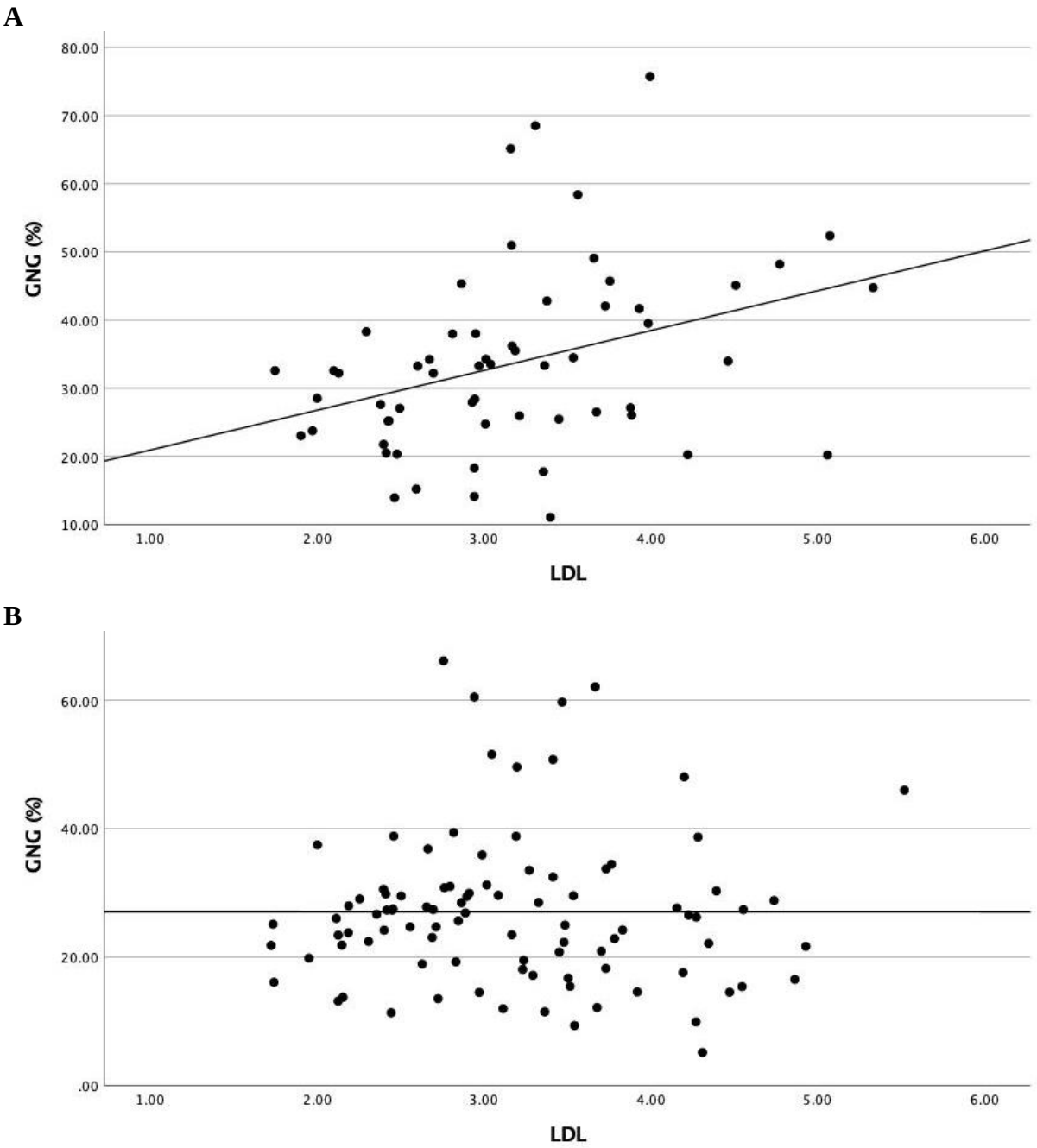

Figure 5. The relationship between GNG and LDL-cholesterol in (A) men and (B) women.
Data is presented with linear line of best fit.

Changes in fasting hepatic DNL and GNG with pharmacological or dietary interventions

As well as analysing hepatic DNL and GNG at baseline across studies, changes after dietary or pharmacological intervention were also explored. For nutritional intervention, participants were studied before and after the consumption of a diet enriched with free sugars for 21 days. After the diet, participants had a significant (p=0.001) increase in fasting hepatic DNL and a significant (p=0.026)

decrease in fasting GNG. This also corresponded to a significant increase in fasting LDL in men (p=0.047, n= 10) but not in women (p= 0.173, n= 6). There was no significant change in fasting plasma glucose and insulin concentrations, nor HOMA-IR.

In the pharmacological intervention, 10 participants were studied before and then after they took metformin (1g twice daily) for 12 weeks. Both fasting DNL and GNG increased by 61% and 26%, respectively, although this was not significant (p=0.203 and p=0.139). There was a small, but significant 2.5% decrease in BMI, with no significant change in fasting plasma glucose or insulin concentrations or IHTG.

Discussion

Obesity is associated with an increased risk of IR as well as metabolic diseases such as T2DM, NAFLD and CVD. Systemic levels of TAG and/or glucose are often raised in these conditions and it has been suggested that this is due to an increase in hepatic DNL and GNG caused by selective hepatic IR. However, this concept has primarily been based on work from rodent models and evidence of selective hepatic IR in humans is sparse. As fasting hepatic DNL is frequently measured, whilst the measurement of hepatic GNG in humans is sparse, we sought to assess the associations of DNL and GNG with markers of IR in humans, in 157 healthy adults.

Contrary to work in rodents, we did not find a signature of selective IR in humans as proposed by Goldstein and Brown[12] where both hepatic DNL and GNG are up-regulated. No association was found between GNG and key markers of IR, such as fasting plasma insulin and glucose or HOMA-IR, nor did participants exhibiting IR have higher GNG than those defined as insulin sensitive. There was a strong positive association between DNL and fasting insulin confirming that this process is upregulated by insulin as previously reported[44], most likely through the SREBP-1c pathway[13,44]. However, DNL showed no association between fasting glucose and although participants with IR had raised DNL compared to the rest of the group, this was not significant. Samuel and Shulman proposed that increased lipolysis from IR peripheral tissues (e.g. adipose) raises the systemic concentration of glycerol, a key precursor for GNG, and hence increases GNG without the liver itself becoming IR[16]. Based on the assumption that an increase in lipolysis would raise plasma NEFA concentrations in proportion to plasma glycerol levels, we found no evidence to support this theory either as no association was found between plasma NEFA levels and GNG.

A novel finding from our work was the sexual dimorphism in fasting hepatic GNG which was not seen for fasting hepatic DNL. By using multiple linear regression, it was found that this was not driven by any single variable, thus it is likely there are several contributing factors. GNG was found to

significantly decrease with increasing age in men but not in women. Thus, taken together, the differences in GNG associated with participant sex and age explain, in part, why our values for GNG were lower than those found in the literature despite using similar methods. Previous studies measuring GNG in humans have been primarily carried out using young, male participants[30,33] which is in contrast to our cohort which was middle-aged and predominantly female. The lack of young participants in our cohort limited our exploration of the change of GNG over age in men. It is hypothesised that the Cahill Cycle may play a role in regulating GNG[45] whereby glucose catabolism into alanine (via pyruvate) within skeletal muscle leads to an increased flux of gluconeogenic substrate to the liver. Therefore, it could be speculated that the age difference in men may be driven by differences in alanine flux which may be higher in those with more skeletal muscle. Based on these observations, we are now undertaking *in vitro* work on hepatocytes to investigate the influence of alanine and other glucogenic precursors (e.g. lactate) on GNG.

Unexpectedly, GNG showed a strong positive association with LDL cholesterol in both the cross-sectional setting and after dietary intervention, which is challenging to explain. Lui et al. previously reported that male mice with heterozygous knock-out of the LDL receptor-related protein 6 (LRP6) exhibited decreased endogenous hepatic glucose production[46] measured by a hyperinsulinemic-euglycemic clamp. Furthermore, statins, which are known for lowering LDL cholesterol, have been shown to increase the risk of diabetes in women but not in men, with one large study suggesting a protective effect in men[47–49]. Taken together, it could be speculated that treatment with statins would reduce GNG and potentially prevent hyperglycaemia which would be of interest to explore for the therapeutic benefits of reducing the risk of multiple metabolic diseases such as NAFLD, T2DM and CVD.

It has been suggested that IHTG accumulation drives the development of IR by increasing DNL and GNG[50] and vice versa[51]. In our study, we found that those with markers of IR had significantly higher IHTG content, however there was no association between IHTG and DNL or GNG. Thus, it is possible that the location and/or composition of the lipid droplets within the hepatocytes is more important in causing metabolic dysregulation than lipid accumulation *per se*.

In the dietary intervention study, a significant increase in DNL and decrease in GNG were observed with no change to fasting insulin and glucose concentrations or HOMA-IR. The proposed mechanism of action for metformin, used in the pharmacological intervention study, is through inhibition of GNG[52–54], however we observed non-significant increases in both GNG and DNL. It is plausible that metformin may increase systemic lactate concentrations[55,56] which may explain the increase in GNG as lactate is a major precursor for this metabolic process. This suggests that metformin may have a different mechanism of action and would be of interest to investigate in larger-scale human studies.

A limitation of this data is that the participant's meal and fasting time were not standardised and this may have affected DNL and GNG. Across all studies, participants were told to have a low-fat meal the evening prior to the study day and to allow for an overnight fast between 8 - 10 hours. As the intrapersonal variability in DNL and GNG is not known it is challenging to determine how large an impact this may have had on the data but for future studies investigating fasting DNL and GNG, participants should be given a standardised meal and fast for a fixed amount of time (e.g. 10 hours) prior to measurements. Furthermore, there was homogeneity within the participant demographic with the majority being white, middle-aged and free from any known disease; it would be of interest to study a cohort across a broader spectrum of age, body fatness and plasma insulin concentrations. Equally it would be of interest to include those with T2DM and to also assess the effects of pharmacological interventions (such as statins and metformin) in these patients.

This study only investigated DNL and GNG in the fasting state and therefore it would be of interest to explore the changes post-prandially. It has been shown that insulin release in response to a meal suppresses GNG and it is postulated that GNG is not adequately suppressed in those with IR in this post-prandial state[57,58]. It has also been suggested that there are various IR phenotypes depending on the tissue affected, with some individuals showing impaired glucose tolerance, whilst others show impaired fasting glucose[59,60].

Taken together, by studying a large cohort of well-phenotyped individuals, we were unable to confirm the metabolic signature of selective hepatic IR identified in rodents, as GNG had no association with DNL or the key markers of IR. Although DNL did exhibit a positive association with insulin and HOMA-IR, it did not have any association with GNG or any other markers of IR. Further work is needed to characterise GNG across a wider range of phenotypes and understand the factors that may impact it, such as diet, fasting length and pharmacological treatments.

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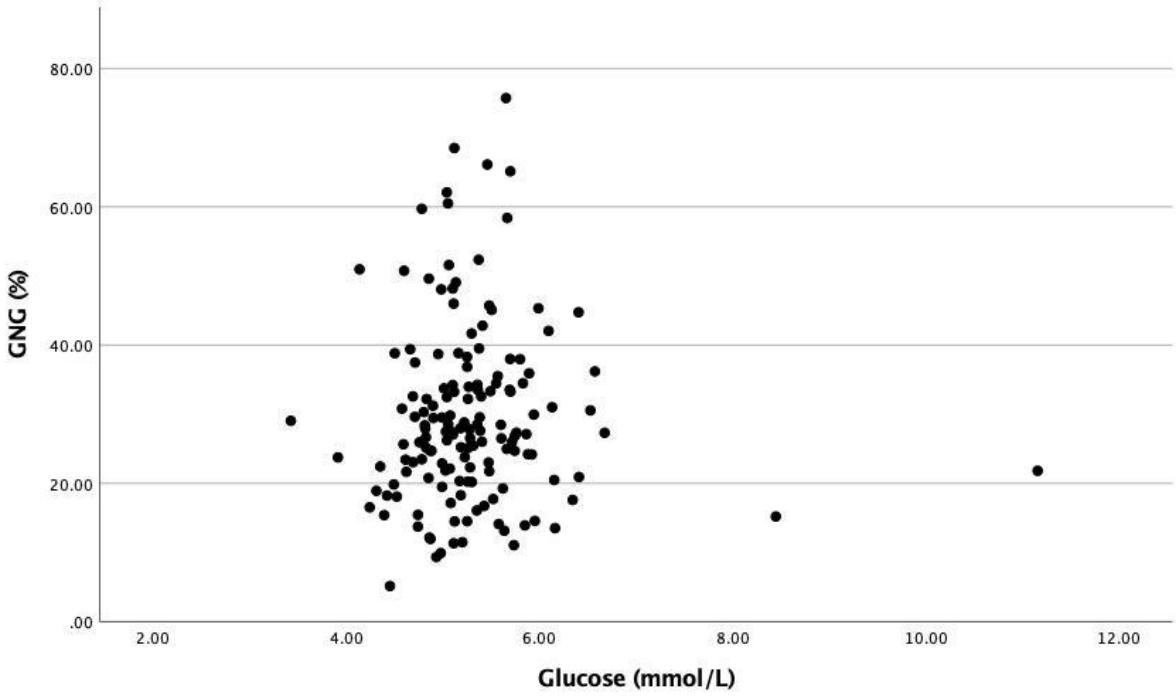
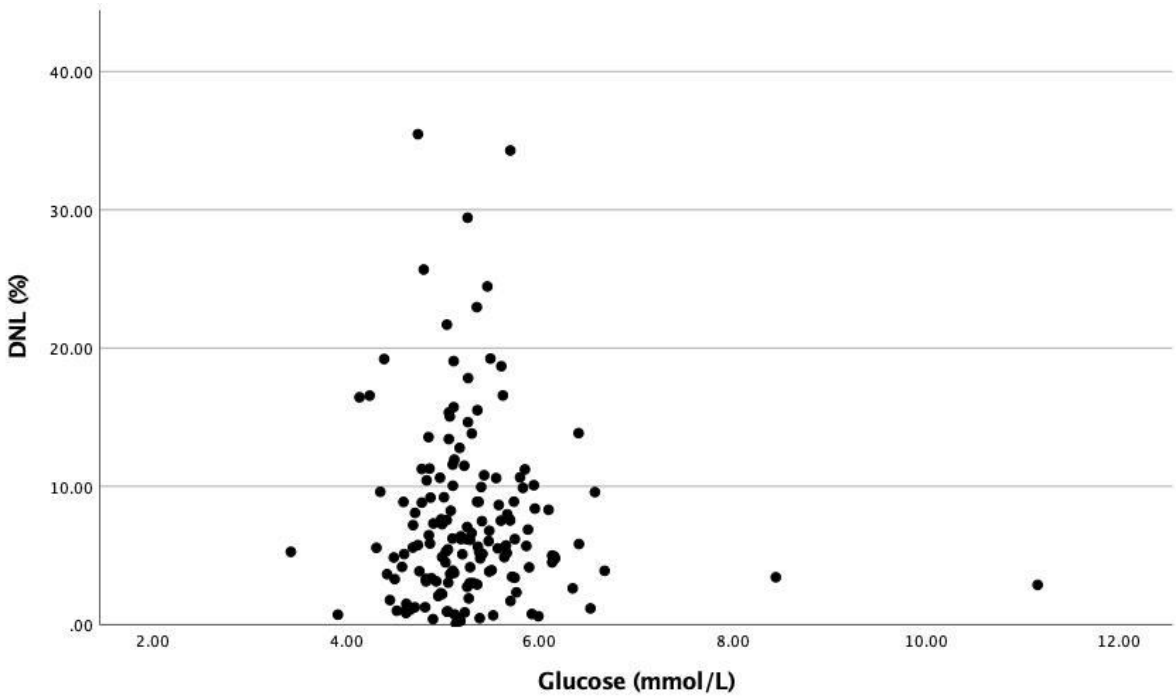
Appendix

A



B



Supplementary Figure 1. Association between fasting plasma glucose and (A) GNG and (B) DNL in men and women.