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Impact of gain-of-function mutations in the low-density lipoprotein receptor-related protein 5 (LRP5) on glucose and lipid homeostasis

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Abstract

Summary—LRP5 loss-of-function mutations have been shown to cause profound osteoporosis and have been associated with impaired insulin sensitivity and dysregulated lipid metabolism. We hypothesized that gain-of-function mutations in LRP5 would also affect these parameters. We therefore studied individuals with LRP5 gain-of-function mutations exhibiting high bone mass (HBM) phenotypes and found that while there was no detected change in insulin sensitivity, there was a significant reduction in serum LDL.

Introduction—Wnt signaling through LRP5 represents a newly appreciated metabolic pathway, which potentially represents a target for drug discovery in type 2 diabetes and hyperlipidemia. Studies in animal models suggest a physiologic link between LRP5 and glucose and lipid homeostasis; however, whether it plays a similar role in humans is unclear. As current literature links loss-of-function LRP5 to impaired glucose and lipid metabolism, we hypothesized that individuals with an HBM-causing mutation in LRP5 would exhibit improved glucose and lipid homeostasis. Since studies in animal models have suggested that Wnt signaling augments insulin secretion, we also examined the effect of Wnt signaling on glucose-stimulated insulin secretion on human pancreatic islets.

Methods—This was a matched case-control study. We used several methods to assess glucose and lipid metabolism in 11 individuals with HBM-causing mutations in LRP5. Affected study participants were recruited from previously identified kindreds with HBM-causing LRP5 mutations and included 9 males and 2 females. Two subjects that were being treated with insulin

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Compliance with ethical standards

Conflicts of interest None.

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for type 2 diabetes were excluded from our analysis, as this would have obscured our ability to determine the impact of gain-of-function LRP5 mutations on glucose metabolism. The mean age of the evaluated study subjects was 55 ± 7 with a mean BMI of 27.2 ± 2.0 . Control subjects were matched and recruited from the general community at an equivalent ratio, with 18 males and 4 females (mean age 56 ± 4 ; mean BMI 27.2 ± 1.0). Study testing was conducted at an academic medical center.

Results—There were no statistically significant differences between affected and matched control populations for HbA1c ($p = 0.06$), eAG ($p = 0.06$), insulin ($p = 0.82$), HOMA-*B* ($p = 0.34$), or HOMA-IR ($p = 0.66$). The mean Insulin Sensitivity Index (ISI) was also similar between control and affected individuals. Total cholesterol ($p = 0.43$), triglycerides (TG) ($p = 0.56$), and HDL ($p = 0.32$) were not different between the same two groups. In a small subset of studied subjects, intramyocellular and hepatic lipid content were similar in the affected individuals and controls when quantified by proton magnetic resonance spectroscopy (MRS). However, the mean value for serum LDL was significantly lower ($p = 0.04$) in affected individuals. In primary human islets, there were no differences between control and Wnt treatment groups for insulin secretion measured as area under the curve (AUC) for first phase ($p = 0.17$) or second phase ($p = 0.33$) insulin secretion.

Conclusions—Although our sample size was small, our data do not support the hypothesis that HBM-causing LRP5 mutations, associated with increased Wnt signaling, improve glucose metabolism in humans. However, it does appear that LRP5 variants may affect LDL metabolism, a major risk factor for coronary artery disease. The molecular mechanisms underpinning this effect warrant further study.

Keywords

Glucose metabolism; Hemoglobin A1c; Insulin secretion; LDL; Lipid metabolism; LRP5

Introduction

The low-density lipoprotein-related protein 5 (LRP5) together with the frizzled family of receptors transduces Wnt signaling via the canonical pathway [1]. Loss-of-function mutations in LRP5 cause profound osteoporosis and fractures [2]. Conversely, mutations in LRP5 that confer resistance to known inhibitors of Wnt signaling, such as sclerostin and Dkk1, result in inherited syndromes of extremely high bone mass [3]. While the role of LRP5 in skeletal homeostasis is now well established, its role in other tissues is unclear. Animal models with both high bone mass (HBM) and low bone mass (LBM)-causing LRP5 mutations, as well as human studies in populations with LBM-causing mutations, suggest a role for LRP5 in glucose and lipid metabolism [4, 5].

Glucose-induced insulin secretion was blunted in pancreatic beta cells isolated from LRP5 knock-out mice. Wnt 3a failed to induce insulin secretion in LRP5-deficient islets, while in wild-type islets, Wnt stimulated glucose-induced insulin secretion [4]. Consistent with these experimental results, a study of kindreds with LBM-causing loss-of-function LRP5 mutations observed that approximately half of these subjects had abnormalities in Hb_{A1c} levels or in oral glucose tolerance tests (OGTT), but this study lacked statistical power to

definitively establish an association between loss of function in LRP5 and insulin resistance [5].

A potential role of LRP5 in lipid metabolism has also been suggested by preclinical experimental data. Fujino et al. demonstrated that LRP5-deficient mice fed a normal diet had normal plasma lipids but developed hypercholesterolemia when fed a high-fat diet [4]. The metabolism of diet-derived triglycerides (TG) was severely impaired in the LRP5-deficient, but not in APoE-deficient mice [6].

Studies in humans have been less conclusive. Several single nucleotide variants in LRP5 have been linked to atherosclerosis [7], increased total cholesterol [8], and increased low-density lipoprotein (LDL) levels [9]. However, Saarinen et al. did not find hypercholesterolemia or elevated triglyceride (TG) levels among kindreds with LBM-causing LRP5 mutations [5]. With regard to insulin sensitivity, human studies have yet to demonstrate any significant associations between genetic variants in LRP5 and glucose metabolism [8–10].

The genetic variants that give rise to the HBM phenotypes have never been included in any of these analyses. Studies in this population could provide insights into the potential role of LRP5 in glucose and lipid homeostasis.

Methods

Study subjects

Nine subjects with a G171V HBM-causing LRP5 mutation and two subjects with an N198S mutation were recruited from previously reported kindreds [3, 11]. Since the skeletal phenotype of patients with G171V and N198S mutations is identical, and since the mechanism by which these allelic variants confer a high bone mass phenotype is the same [3, 12–14], we have pooled the data from both kindreds because there is no reason to expect that the effects of these two variants on Wnt signaling in metabolic tissues would vary. Each kindred member was assigned to two unrelated control subjects matched for sex, age, race, and body mass index (BMI). Control subjects were recruited from the Yale New Haven Hospital community and from colleagues and associates of the authors. All control and study subjects provided written informed consent for this study. Hb_{A1c} and insulin were measured in all participants. Three affected subjects agreed to undergo an OGTT, and two of these subjects underwent magnetic resonance spectroscopy (MRS) studies. The data from the three subjects who had OGTTs was compared to data previously obtained in 54 control individuals [15, 16]. These studies were approved by the Yale Human Investigation Committee.

Plasma metabolite concentrations

Fasting blood samples were drawn on the Yale New Haven Hospital Research Unit. Hb_{A1c} was measured using Premier Hb9210 HPLC according to the manufacturer's instructions (Trinity Biotech; Jamestown, NY).

Oral glucose tolerance test

Whole body insulin sensitivity was assessed with a standard 3-h 75-g oral dextrose challenge (Glucola®). Plasma glucose concentrations during the OGTT were measured using an autoanalyzer. Insulin concentrations were measured using a Linco RIA kit (EMD Millipore; Billerica, MA).

Lipid panel

Total cholesterol (TC), high-density lipoprotein (HDL), and TG concentrations were measured using an autoanalyzer. LDL concentrations were calculated from TC, HDL, and TG with the Friedewald formula [17].

¹H magnetic resonance spectroscopy measurements of intramyocellular, extramyocellular, and hepatic lipid content

Proton intramyocellular (IMCL) and extramyocellular (EMCL) and hepatic lipid content were measured using ¹HMRS at 4 Tesla as previously described [18, 19].

Human islet perfusion

Human islets were obtained from the University of Alberta Islet Core, Alberta Diabetes Institute, Edmonton [20]. Islets were cultured in complete CMRL media [21] at 37 °C with 5% CO₂, 95% air. Prior to perfusion, 160 islets were pretreated with Wnt 3 a (75 ng/mL), and another 160 islets without Wnt (control) for 19 h. At the beginning of the experiment, islets were loaded into columns (40 islets/column condition, $n = 4$) of an automated perfusion system (Biorep Technologies) and perfused with DMEM (D5030, Sigma) supplemented with NaHCO₃, 2.5 mM glucose, 4 mM glutamine, 10 mM HEPES, and 0.2% FFA-free BSA for 45 min in order to equilibrate [22]. Islets were then perfused with basal glucose (2.5 mM) $\pm$ Wnt 3a followed by two stimuli steps, 9 mM glucose $\pm$ Wnt 3 a (45 min), and 30 mM KCl $\pm$ Wnt 3 a, the latter to evaluate insulin secretion independent of glucose metabolism. Islets were perfused between each stimuli step with 2.5 mM glucose (15 min). Throughout the perfusion protocol, 100 μ l of perfusate was collected per minute from each column. Insulin levels in the perfusate were measured with an ELISA (80-INSHU-E01, ALPCO Diagnostics, Salem, NH) and normalized to the DNA content of the cell layer (P7589, Molecular Probes).

Calculations

The Insulin Sensitivity Index [23], HOMA-*B*, and HOMA-IR [24] were calculated as previously reported. Statistical differences were analyzed using the unpaired Student's *t* test (GraphPad Prism version 6.00 for Windows, GraphPad Software; La Jolla California USA, www.graphpad.com). Data are presented as mean $\pm$ standard error of the mean (SEM). Differences were considered statistically significant when $p < 0.05$.

Results

Hb_{A1c} is not significantly different in individuals with HBM mutations in LRP5

The relevant demographic and baseline biochemical parameters of the affected individuals and controls are summarized in Table 1. Two affected male subjects, one 80 and one 90 years of age, had insulin-dependent diabetes and were excluded from analysis (Table 1). Based on available literature, the hypothesis tested in this study was that patients with LRP5 gain-of-function mutations would evidence a more robust insulin response to a dietary glucose challenge and/or have greater insulin sensitivity. Consequently, we felt that prior treatment with insulin for type 2 diabetes would obscure our ability to test this hypothesis. The mean age of our analyzed cohort was 55 ± 7 years of age.

Thus, the study included the 9 affected individuals and 18 matched-control subjects. There was no significant difference in mean Hb_{A1c} levels between the affected ($5.8 \pm 0.3\%$) and the control groups ($5.4 \pm 0.5\%$; $p = 0.06$), despite a power of 99% to detect a 1.0% difference and 80% to detect a 0.6% difference, which would be considered clinically significant (Fig. 1). There were also no differences in circulating levels of insulin in the affected and control populations ($p = 0.82$), HOMA-*B* ($p = 0.34$), or in HOMA-IR ($p = 0.66$). The ISI, which mostly reflects whole body insulin sensitivity, was similar between the control ($n = 54$, ISI 4.66 ± 0.22 dL/min per microunit/mL) and the affected study subjects ($n = 3$, ISI 3.12, 6.62, 6.54 dL/min/ μ U/mL).

Three affected individuals underwent oral glucose tolerance testing. When compared to published controls from previous studies, plasma glucose, insulin, and c-peptide levels were not consistently different from the control group, suggesting no difference in insulin production or in insulin sensitivity in this subgroup. As was the case in the group as a whole, these three subjects had normal fasting plasma glucose concentrations and normal glucose tolerance. Finally, both liver TG and IMCL content in both subjects who underwent MRS were not significantly different from those of matched controls (Supplemental Table 1).

LDL cholesterol levels are lower in individuals with mutated LRP5

As shown in Fig. 2, mean TC, TG, and HDL were similar in affected and control subjects. However, mean LDL in affected subjects was significantly lower compared to controls ($p = 0.04$).

Wnt does not augment glucose-stimulated insulin secretion in primary human islets

We next examined the effect of Wnt on insulin secretion in primary human islets, which are known to have robust expression of LRP5 [25]. We perfused human islets with Wnt 3a under stimulatory glucose and non-stimulatory conditions. There were no detectable differences between control and Wnt treatment groups for insulin secretion measured as area under the curve (AUC) for first-phase ($p = 0.17$) or second-phase ($p = 0.33$) insulin secretion.

Discussion

Wnt signaling through LRP5 represents a newly appreciated metabolic pathway, which based on animal work, could be a potential target for drug discovery in type 2 diabetes and hyperlipidemia. In pre-adipocytes, Wnt/LRP5 and insulin signaling have been shown to interact, exerting a positive effect on insulin signaling [26]. Studies in rodents support the conclusion that LRP5 mediates glucose and lipid metabolism [4, 6]. Based on these data, it would seem likely that alterations in LRP5 signaling in humans would also alter glucose metabolism. However, prior to the current report, there were no data in patients with augmented LRP5 signaling. In this context, we studied individuals with HBM-causing mutations in LRP5.

Our data suggest that individuals with HBM-causing LRP5 mutations are not more insulin-sensitive than matched control subjects. There were no significant differences in Hb_{A1c}, plasma glucose, insulin, c-peptide levels, or ISI, Homa-*B*, and Homa-IR scores. The fact that the time-dependent changes in blood levels of glucose c-peptide and insulin in the three affected individuals who underwent OGTTs did not vary widely from the changes induced in normal individuals suggests that, in humans, augmented Wnt signaling mediated by LRP5 gain-of-function mutations does not affect glucose metabolism (Supplemental Fig. 2). Two affected individuals underwent MRS of liver and skeletal muscle. We hypothesized that one mechanism by which enhanced LRP5 signaling in affected individuals could result in improved glucose metabolism was by improved insulin sensitivity resulting from reduced IMCL. There exists substantial literature on assessing IMCL as a biomarker of insulin sensitivity [19, 23, 27]. In both MRS-tested subjects, IMCL was not significantly different compared to the control group. Given our small sample size, it is possible that this may be a type 2 error. However, insulin sensitivity in these same two affected individuals was not different from that of the matched controls. Two of the 11 subjects had type 2 diabetes, which represents a prevalence of 18% within our cohort. This prevalence rate is within the range of the estimated prevalence of type 2 diabetes in the general population for individuals whose ages are similar to those of our study population. In particular, the CDC reports that 16.2–25.9% of individuals in the age range from 45 to 65+ have type 2 diabetes [28]. Therefore, we do not believe that the gain-of-function LRP5 variants in these two individuals played a pathogenic role in their type 2 diabetes, considering that the mean age of our entire cohort was 60 ± 7 years of age.

Although Wnt signaling augments insulin release from murine islets [4], the effect of Wnt signaling in human islets has not been reported. Wnt treatment did not change human insulin production under basal or glucose-stimulated culture conditions. Wnt3a was used to activate Wnt signaling in these studies because prior work in isolated murine islets had demonstrated that activation of canonical Wnt signaling using this Wnt ligand augmented insulin release [4]. The reason our findings differ from those in the mouse is not clear. One possibility is that murine and human islets respond differently to Wnt because the micro-architecture and cellular composition of the murine islet is different from that of the human islet [29], thereby conditioning different islet cell responses to cytokines and hormones.

As was the case with studies on glucose metabolism in humans, prior studies of lipid metabolism in patients with LOF mutations in LRP5 have not corroborated the findings in LRP5 knock-out mice [5, 10]. Our analysis of lipid metabolism in the affected study population excluded individuals on lipid-lowering drugs and controlled for gender, age, and BMI. Total cholesterol, TG, and HDL were not different between affected and control populations. However, LDL was significantly lower in the affected subjects. It should be noted that we excluded three patients on lipid-lowering drugs from this analysis. Had they been included, the difference between controls and affected individuals would have been artifactually greater. The fact that LDL levels were significantly lower in affected individuals is particularly interesting, as the LRP5 receptor binds ApoE-containing lipoproteins in vitro [30], and may mediate cholesterol metabolism independently of both the ApoE receptor and LDL receptor [6, 31]. One proposed mechanism for LRP5's role in cholesterol catabolism suggests that LRP5 stimulates TG hydrolysis via its interaction with Dkk [6]. If this were the case, we might expect to see lower levels of triglycerides in our study of humans. However, we found no difference in plasma TG levels as shown in Fig. 2. Similarly, ¹H MRS studies of liver and muscle found no consistent difference in TG content between affected and control groups.

Our study has several strengths. Given how rare high bone mass-causing variants of LRP5 are in the general population, this study represents a relatively large number of affected individuals. Further, we used several approaches, both in vivo and in vitro, to interrogate the relation between LRP5 signaling and fuel metabolism. However, although our data does not support the hypothesis that augmented LRP5 signaling improves glucose homeostasis, our data set is too limited to consider our findings as definitive. For example, it has been shown that LRP5 variants track with bone mass in the general population [32]. The relationship of those variants to glucose homeostasis has not been explored and perhaps could uncover associations missed in our study.

In summary, given the caveats just noted, our data do not support the hypothesis that LRP5 improves glucose metabolism in individuals exhibiting HBM-causing LRP5 mutations and enhanced Wnt signaling. However, it does appear that there may be an effect on LDL metabolism, a major risk factor for coronary artery disease. The molecular mechanisms underpinning this latter effect warrant further study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Fig. 1.
Hb_{A1c} (%) in study participants. Hb_{A1c} values are not significantly different in LRP5 affected individuals and age, gender, and BMI matched controls ($p = 0.06$)

Fig. 2.

Lipid levels in individuals with a HBM causing LRP5 allelic variant and control subjects. Total cholesterol ($p = 0.43$), triglycerides ($p = 0.56$), and HDL ($p = 0.32$) were similar in affected individuals and age, gender, and BMI-matched controls. LDL was significantly lower ($p = 0.04$) in affected individuals. Three affected subjects (subjects #1, 3, and 5) were not included in the analysis because they were being treated with lipid-lowering agents

Table 1

Baseline demographic information—affected individuals and matched

Study subject no.	Kindred ^a (A,B,C)	Affected (A)/not-affected (N)	Age	Sex (M/F)	BMI (kg/m ²)	LRP5 mutation	HbA1c (%)	Insulin(fasting) µU/mL	Cholesterol	Triglycerides	HDL	LDL-calculated	OGTT
1	A	A	90	M	22.3	G171V	^b 6.4	5.5	N/A	N/A	N/A	N/A	No
2	A	A	16	M	20.8	G171V	5.6	8.0	115	49	40	65	No
3	A	A	51	M	35.0	G171V	8.2	14.9	N/A	N/A	N/A	N/A	No
4	A	A	37	M	34.0	G171V	5.2	17.8	180	112	58	100	No
5	A	A	62	M	29.4	G171V	5.6	10.6	N/A	N/A	N/A	N/A	Yes
6	B	A	78	M	24.8	G171V	5.4	9.9	185	66	71	101	No
7	B	A	50	M	32.7	G171V	5.9	24.4	163	104	45	97	No
8	B	A	63	M	26.7	G171V	5.5	5.8	149	44	68	72	Yes
9	B	A	80	M	34.7	G171V	^b 6.8	12.1	143	140	49	66	No
10	C	A	80	F	21.2	N198S	5.7	12.4	209	132	101	82	No
11	C	A	55	F	20.1	N198S	5.4	7.0	212	96	80	113	Yes
12	-	N	51	M	30.5	-	5.1	43.1	161	156	33	97	-
13	-	N	62	M	25.9	-	5.4	14.3	147	132	34	87	-
14	-	N	79	M	23.6	-	5.1	9.7	170	59	60	98	-
15	-	N	53	F	22.6	-	5.4	7.8	223	82	72	135	-
16	-	N	76	M	25.1	-	5.9	13.4	125	39	50	67	-
17	-	N	54	F	25.8	-	5.7	9.0	218	91	66	134	-
18	-	N	59	M	31.1	-	5.4	5.6	152	110	42	88	-
19	-	N	61	M	23.6	-	5.6	7.1	206	55	65	130	-
20	-	N	58	M	30.4	-	5.5	17.5	226	148	61	135	-
21	-	N	56	M	28.7	-	5	6.9	166	79	48	102	-
22	-	N	41	M	30.6	-	5.4	8.6	218	55	57	150	-
23	-	N	35	M	30.6	-	5.2	28.0	197	173	44	118	-
24	-	N	78	F	23.5	-	5.6	8.0	185	41	78	99	-
25	-	N	82	F	32.3	-	5.4	5.8	225	80	82	127	-
26	-	N	90	M	24.2	-	5.7	16.1	194	198	49	105	-
27	-	N	53	M	35.2	-	5.4	6.5	299	370	58	167	-
28	-	N	83	M	29.6	-	5.4	12.3	N/A	N/A	N/A	N/A	-
29	-	N	79	M	40.4	-	6.5	16.8	N/A	N/A	N/A	N/A	-
30	-	N	93	M	22.0	-	4.9	7.4	107	45	40	58	-
31	-	N	62	M	29.9	-	5.4	23.0	N/A	N/A	N/A	N/A	-

Study subject no.	Kindred ^a (A,B,C)	Affected (A)/not-affected (N)	Age	Sex (M/F)	BMI (kg/m ²)	LRPS mutation	HbA1c (%)	Insulin(fasting) µU/mL	Cholesterol	Triglycerides	HDL	LDL-calculated	OGTT
32	-	N	21	M	20.7	-	5.3	13.7	155	62	40	103	-
33	-	N	20	M	20.0	-	5.4	7.1	143	48	57	76	-

^a Pedigrees of kindreds A and B are presented in Supplementary Fig. 1

^b Study subjects being treated for type 2 diabetes