Glucokinase Polymorphism Interacts with Intakes of Low-Fat Dairy Foods to Influence Variables Related to Glucose-Insulin Homeostasis

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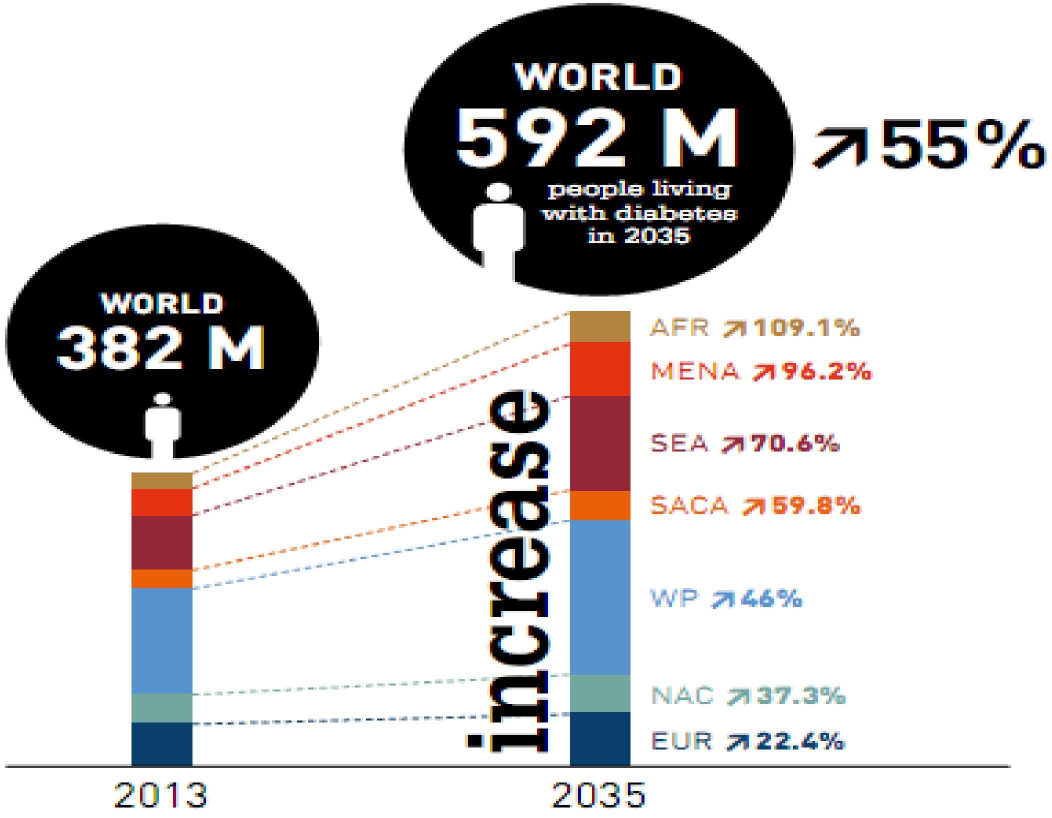
Background and aim: Meta-analyses studies have associated the consumption of high-fat (HF) and low-fat (LF) dairy products to improvements in glycaemia as well as risk factors associated to type 2 diabetes (T2D). However, a recent review of dairy product intervention studies on insulin sensitivity demonstrated mixed results: 4 studies showing improved insulin sensitivity, 1 showing worsened values, and 5 showing no effect. This may be partly due to genetic variability in the population studied. Glucokinase (GCK) is a key regulator of glucose disposal and storage in both liver and pancreatic beta-cells, and responds to increases in circulating glucose concentration by initiating a signalling cascade that results in insulin secretion. Studies have associated single-nucleotide polymorphisms (SNPs) in *GCK* gene with impaired glucose regulation and increased risk of T2D. The objective of the study is to investigate the gene–diet interaction effects between SNPs within *GCK* gene and dairy product consumption on variables related to glucose-insulin homeostasis. **Materials and Methods:** Dietary data using a validated food frequency questionnaire together with fasting blood samples were collected from 210 healthy French Canadians. Dairy products were evaluated as HF (>2 %) and LF (<2 %) servings per day, then dichotomized into high- and low- intake based on population median. Insulin resistance was calculated using the homeostatic model of the assessment of insulin resistance (HOMA-IR). Thirteen SNPs covering 86% of the known genetic variability within *GCK* gene were genotyped using TAQMAN methodology. **Results:** More than one-third (42%) of individuals did not meet the minimum recommendations for dairy intake from Canada's Food Guide (<2 servings of dairy products/day). LF dairy intakes were inversely correlated with fasting plasma glucose level (r=-0.1957, *P*=0.0048), adjusted for age, sex and BMI. No correlations were observed between dairy intakes and plasma insulin or HOMA-IR levels. The ANOVA model was used to test for the effects of the *GCK* genotypes, dairy intake, and the genotypes by dairy intake interaction on glycemic parameters, adjusted for age, sex and BMI. No interaction effects were observed with HF dairy products. We identified a significant interaction between the rs758989 with LF dairy intake on HOMA-IR (*P* interaction=0.0006). Specifically, homozygotes of the A allele of rs758989 together with LF dairy products (<1.22 portions/day) had a higher HOMA-IR compared with other genotypes or high dairy consumers. **Conclusions:** These results indicate that the intake of LF dairy products may influence glucose-insulin homeostasis in individuals with specific SNPs related to the risk factors of T2D. Replication studies are needed. **Grant Acknowledgement:** CIHR (MOP229488) and FRQ-S.

Diabetes is a **huge and growing problem**, and the costs to society are **high and escalating**



382 million people have diabetes

By **2035**, this number will rise to **592 million**

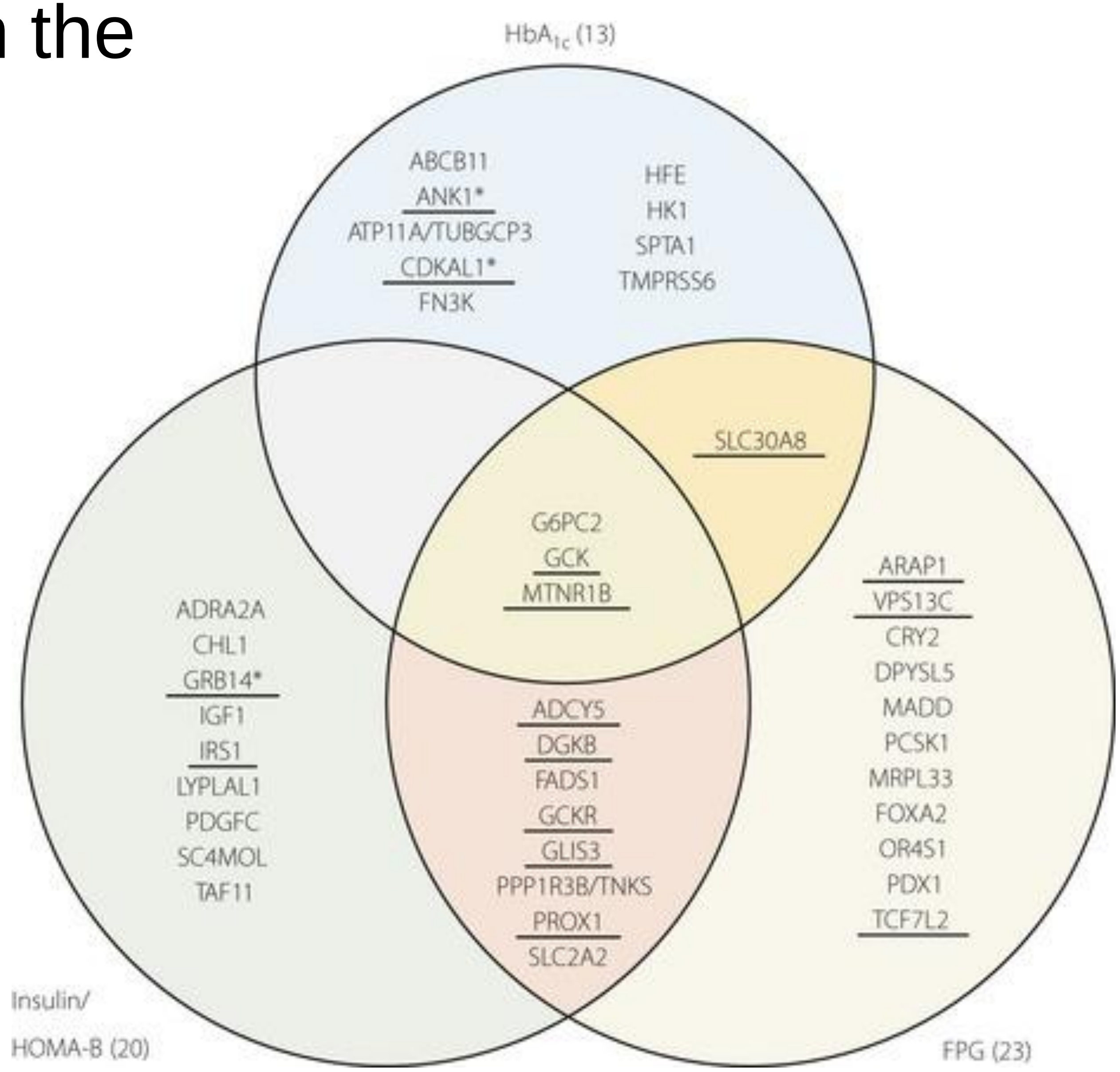
Type 2 diabetes

- Insulin resistance
- Lifestyle factors
- Usually adults

International Diabetes Federation, *2014. http://www.idf.org/diabetesatlas*

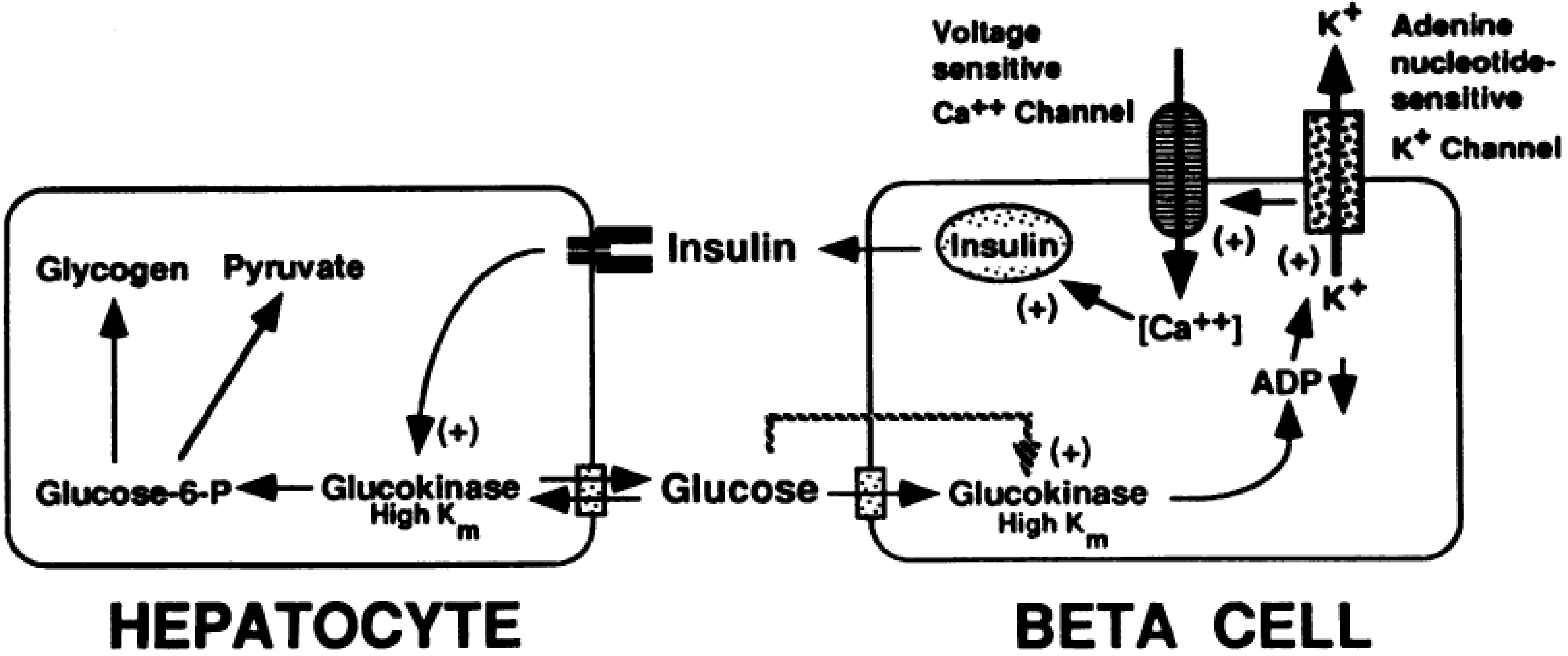
Polymorphisms associated with the risk of type 2 diabetes

- Large-scale genome-wide association (GWA) studies have successfully identified genetic loci associated with type 2 diabetes:
  - fasting plasma glucose (FPG)
  - insulin
  - homeostasis model assessment of β-cell function (HOMA-B)
  - glycated hemoglobin (HbA_{1c})



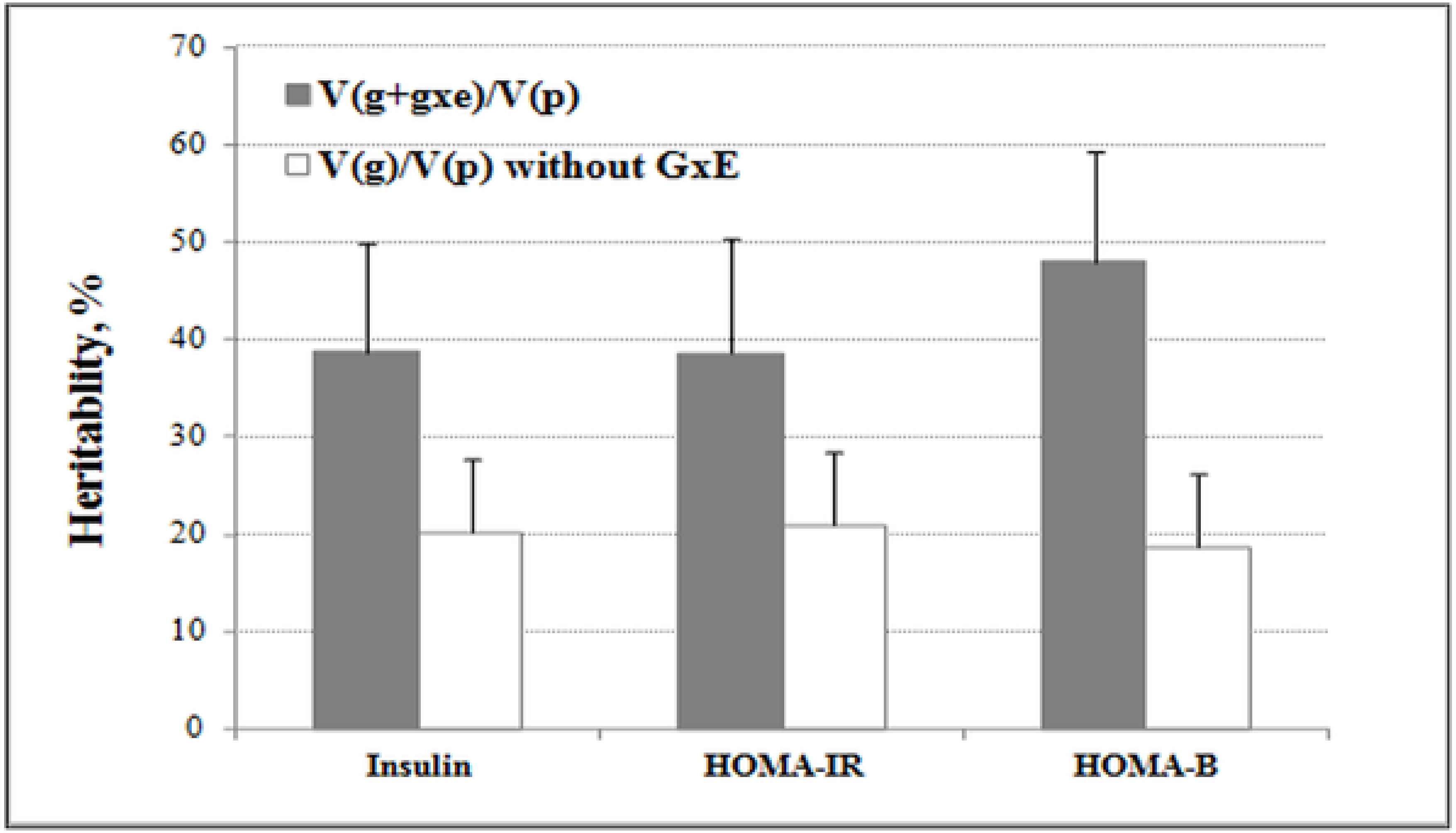
Kato N. *J Diabetes Investig. 2013*

Glucokinase (*GCK*) and glucose homeostasis



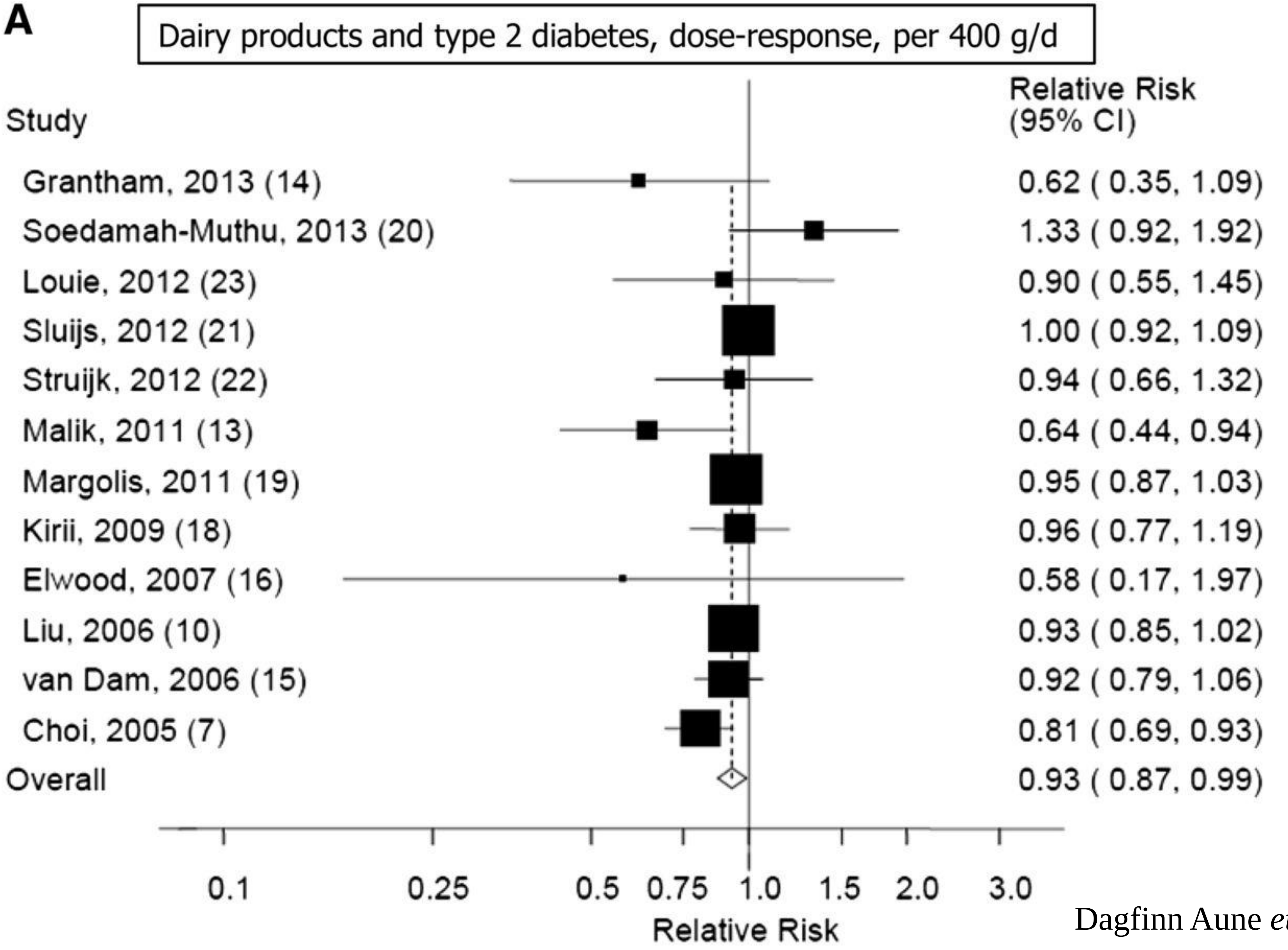
Matschinsky et al., *J. Clin. Invest.* 1993

Proportion of risk due to genetic factors (%) of traits related to type 2 diabetes



Zheng J-S, et al. *PLoS ONE* 2013

Intake of total dairy products and risk of type 2 diabetes



Dagfinn Aune *et al.* Am J Clin Nutr 2013

Randomised controlled intervention studies that assessed the impact of altering dairy consumption on insulin sensitivity

Reference	Study design	Study participants	Intervention	Effects of intervention
Rideout et al. 2013	Randomised crossover study of two 6-month interventions	23 individuals aged 18-75, BMI 18.5-35 kg/m2	4 serves dairy/day compared to no more than 2 serves dairy/day	High dairy ↓ insulin, **improving HOMA-IR**
Zemel et al. 2005	Randomised parallel study of two 24-week interventions	34 African-Americans aged 26-55, BMI 30-40 kg/m2	3 serves dairy/day or <1 serve dairy/day	↓ fasting insulin in dairy group
Stancliffe et al. 2011	Randomised parallel study of two 12-week interventions	40 individuals, BMI 25- 39.9 kg/m2 with >3 components of MetS	>3.5 serves dairy/day compared to <0.5 serves dairy/day	Adequate dairy ↓ insulin, **improving HOMA-IR** compared to control
Pal et al. 2010	Randomised parallel study of three 12-week interventions	70 individuals aged 18-30, BMI 25-40 kg/m2	54 g of whey protein, casein protein or glucose control	Whey ↓ insulin compared to baseline and to control

Adapted from Turner *et al.*, 2014 NMCD

Randomised controlled intervention studies that assessed the impact of altering dairy consumption on insulin sensitivity (2)

Reference	Study design	Study participants	Intervention	Effects of intervention
Crichton et al. 2012	Randomised crossover study of two 6-month interventions	61 participants aged 18-75, BMI >25 kg/m2	4 serves dairy/day compared to <1 serve dairy/day	↔ glucose or insulin
Hoppe et al. 2009	Randomised crossover study of two 10-day interventions	11 healthy males aged 22-29	2.5 L low-fat milk/day or 2.5 L Coca-Cola/day	↔ between groups for insulin or glucose, or from baseline
Benatar et al. 2013	Randomised parallel study of three 1-month interventions	176 healthy normal-weight participants	Increase dairy by 2-3 serves/day; maintain usual dairy intake; eliminate dairy	↔ glucose or insulin
Wennersberg et al. 2009	Randomised parallel study of two 6-month interventions	113 individuals aged 30- 65 with at least 2 components of MetS, with habitual dairy intake <2 serves/day	<2 serves dairy/day or 3-5 serves dairy/day	↔ insulin in milk group but ↑ insulin in control resulting in ↑ HOMA-IR
Van Meijl et al. 2011	Randomised crossover study of two 8-week interventions	35 individuals aged 18-70, BMI >27 kg/m2 with habitual dairy intake <500 g/day	500 mL low-fat milk and 150 g low-fat yoghurt/day compared to 600 mL fruit juice and 43 g fruit biscuits	↔ glucose or insulin

Adapted from Turner *et al.*, 2014 NMCD

Objective of the study

- To investigate the gene–diet interaction effects between SNPs within *GCK* gene and dairy product consumption on variables related to glucose-insulin homeostasis.

Methods: Subject Recruitment

Men and pre- and postmenopausal women who where:

1- overweight (BMI >25 kg/m^2)

2- 18–50 years old

3- Not taking glucose-lipids lowering medications

Da Silva *et al.*, Appl. Physiol. Nutr. Metab. 2014

Methods: Laboratory

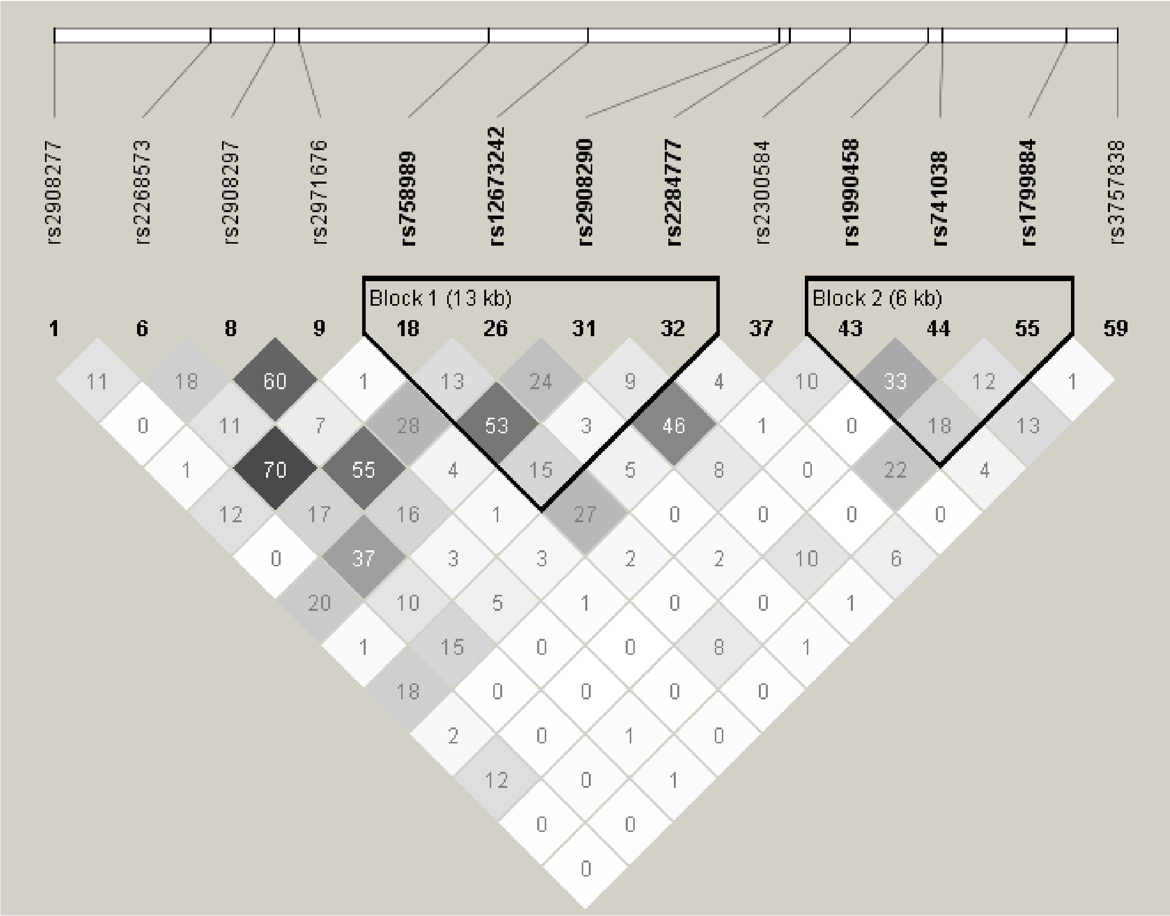
Biochemical parameters

- Fasting insulinemia was measured by radioimmunoassay
- Fasting glucose concentrations were enzymatically measured
- Homa-ir

DNA extraction and genotyping

- The SIGMA GenElute Gel Extraction Kit (Sigma-Aldrich Co. St.Louis, Missouri, USA) were used to extract genomic DNA
- SNPs in *GCK* were identified using the International HapMap Project SNP database, Tagger procedure in Haploview V4.2 was used to determine tag SNPs (tSNPs) using a minor allele frequency (MAF) > 5% and pairwise tagging ($r^2 \geq 0.8$)
- Genotypes were determined using a 7500 FAST RT-PCR System and analyzed using ABI Prism SDS version 2.0.5 software (Applied Biosystems, Foster City, CA, USA)

Linkage disequilibrium (LD) plot of tSNPs within *GCK* gene



Thirteen SNPs covering 86% of the known genetic variability within *GCK* gene were genotyped using TAQMAN methodology

Bouchard-Mercier et al, *Genes Nutr* 2014

Methods: Dietary data

- 91-item validated FFQ (Goulet et al., 2004) administered by a registered dietitian
  - Analysed using the Nutrition Data System for Research software
- 3 subgroups of dairy products:
  - **Low-fat dairy product subgroup** included < 2 % - fat dairy products
  - **High-fat dairy product subgroup** included > 2 % - fat dairy products
  - **Total dairy product** intake was defined as the sum of low-fat and high-fat dairy intakes
- Dichotomized into high- and low- intake based on population median.

Composition of the low-fat and high-fat dairy products and portions

Dairy product subgroup	Products	Fat content	Equivalent to 1 portion
Low-fat	Milk	Skim, 1% or 2%	250 mL
	Yogurt	Skim, 1% or 2%	175 g
	Frozen yogurt	<2%	175 g
	Cottage cheese	0%–2%	250 mL
High-fat	Milk	Whole	250 mL
	Cheese	All kinds	50 g
	Yogurt	>2%	175 g
	Cottage cheese	2%–4%	250 mL

Da Silva *et al.*, Appl. Physiol. Nutr. Metab. 2014

Statistical methods

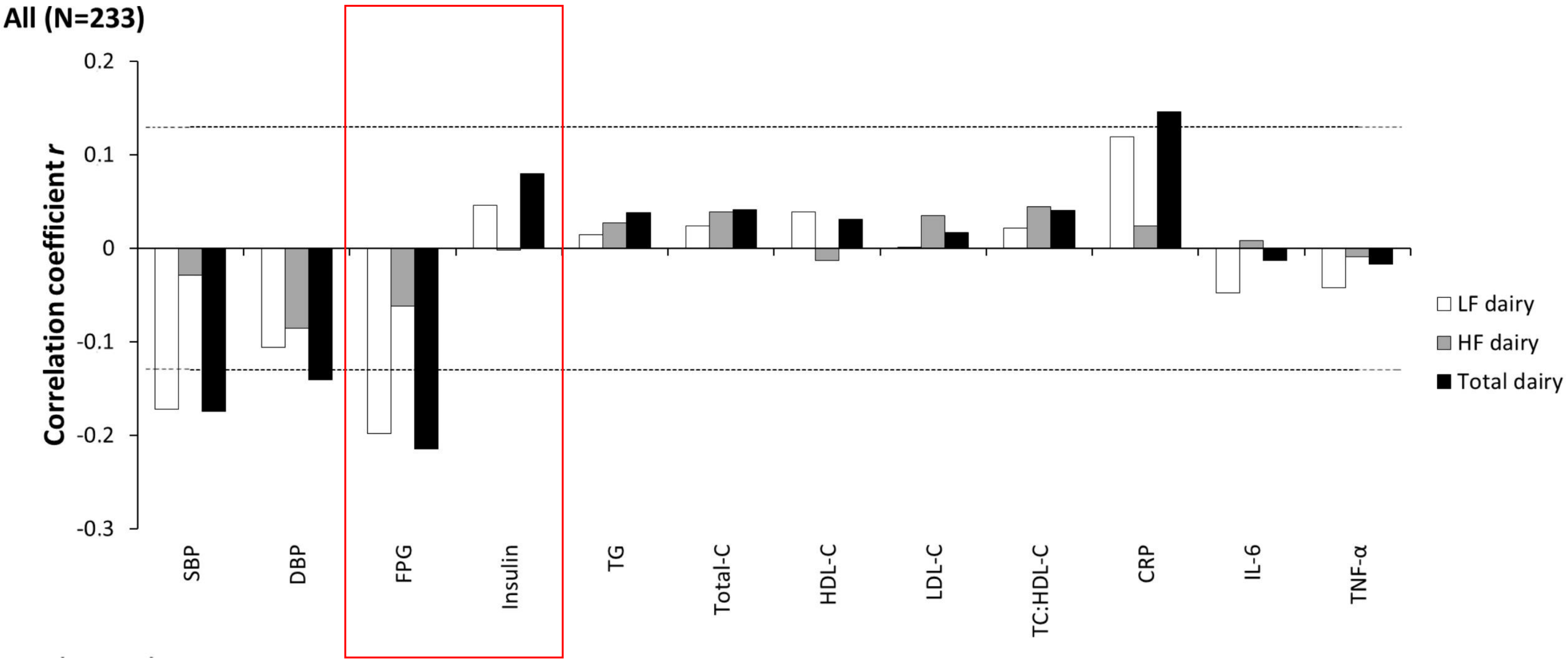
- **Correlations:**
  - Parameters were assessed either by Pearson’s or Spearman’s correlation coefficients, depending on the normality of the variables. Correlations coefficients were adjusted for age and BMI
- **Genotypes:**
  - Hardy-Weinberg equilibrium was tested with the Allele Procedure
  - Distribution of alleles in the present study cohort was compared with the Caucasian population and the Fisher Exact Test
- **Gene-diet interactions:**
  - Variables non-normally distributed were logarithmically transformed (insulin)
  - ANOVA was used to test for the effects of the genotype, the dairy intake (high or low) and the genotype by dairy intake interaction effect on each variable when adjusted for the effects of age, sex and BMI
- A statistical *p*-value was defined as $p \leq 0.05$
  - When significant differences were found, a pairwise comparison was performed in a global analysis (Tukey tests; significance $p \leq 0.05$)
  - SAS statistical software, version 9.2 (SAS Institute Inc.)

Results: Subjects characteristics

	All (N=233)	Men (N=105)	Women (N=128)	p*
Age (y)	30.5±8.7	30.9±8.2	30.2±9.1	0.56
Body mass index ($kg \cdot m^{-2}$)	27.7±3.7	27.3±3.5	28.0±3.8	0.12
Waist/hip ratio†	0.86±0.06	0.89±0.06	0.84±0.06	<0.0001
Waist circumference (cm)	93.2±10.5	93.8±11.2	92.7±9.9	0.47
Systolic blood pressure (mm Hg)	112.2±11.7	118.3±11.4	107.2±9.3	<0.0001
Diastolic blood pressure (mm Hg)	68.1±8.7	68.6±8.2	67.6±9.0	0.45
Fasting plasma glucose ($mmol \cdot L^{-1}$)†	4.94±0.46	5.03±0.45	4.87±0.46	0.01
Insulin ($pmol \cdot L^{-1}$)‡	83.3±49.1	82.3±57.7	84.1±41.1	0.26
HOMA-IR‡	2.66±1.79	2.68±2.21	2.65±1.37	0.89

Da Silva *et al.*, Appl. Physiol. Nutr. Metab. 2014

Correlations between low-fat (LF), high-fat (HF), and total dairy intakes and metabolic risk factors adjusted for age and BMI (n= 210)



Da Silva *et al.*, Appl. Physiol. Nutr. Metab. 2014

Gene-diet interactions: rs758989* total dairy intake adjusted for age, sex and BMI (n= 210)

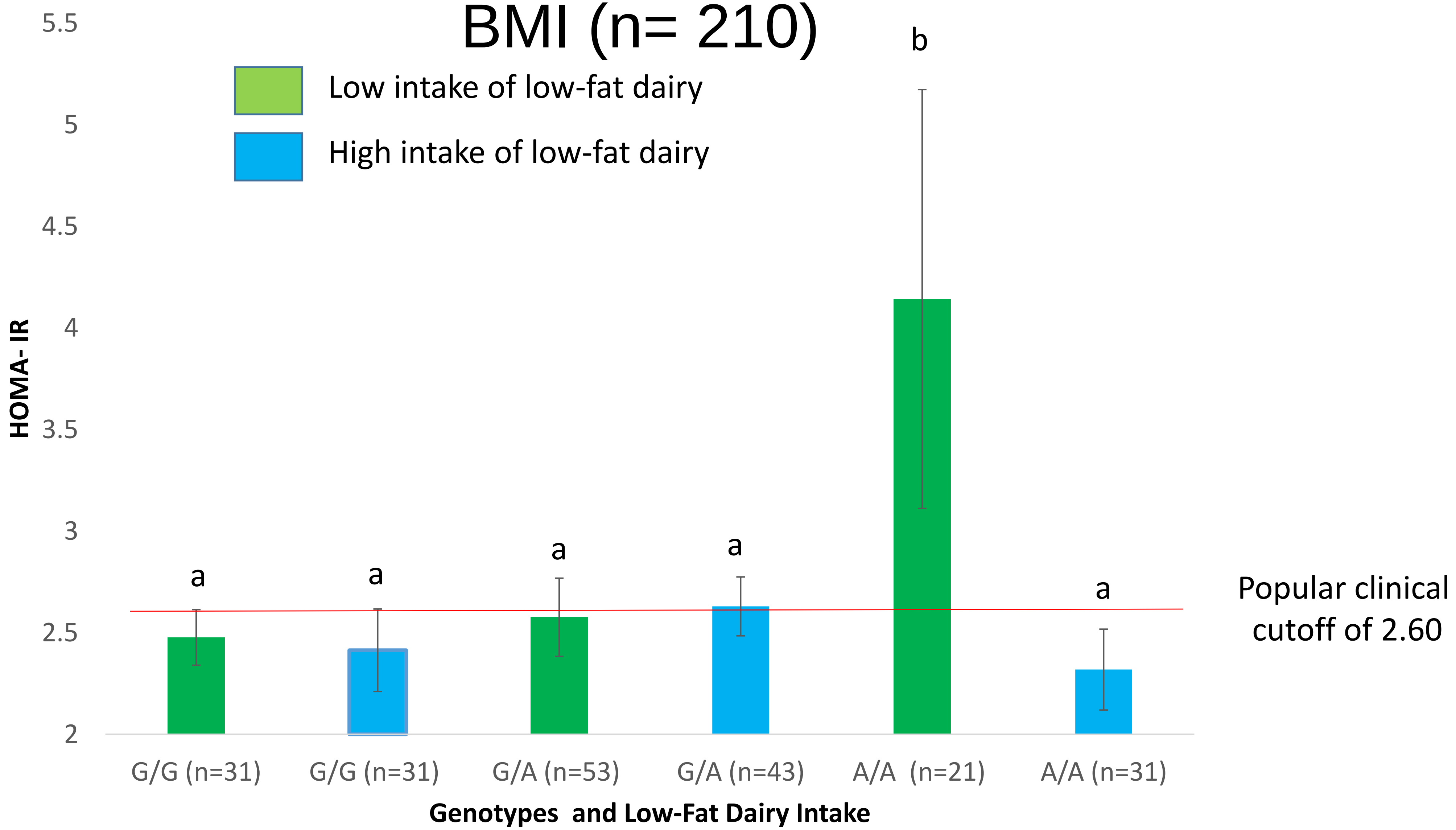
Total dairy intake (median: 2.17 portions/d)	G/G		G/A		A/A		P value Genotype	P value High-fat dairy	P value interaction
	Low (n=28)	High (n=34)	Low (n=52)	High (n=44)	Low (n=25)	High (n=27)			
Glucose	4.99±0.34	4.92±0.52	5.03±0.46	4.87±0.41	5.06±0.46	4.81±0.50	0.5067	0.5482	0.4880
Insulin	70.89±15.75	83.06±27.06	83.08±42.77	79.39±25.38	114.74±108.22	75.11±34.03	0.7118	0.8607	0.1883
HOMA-IR	2.27±0.54	2.58±1.17	2.70±1.45	2.49±0.86	3.89±4.35	2.33±1.12	0.1910	0.1897	0.0028

Gene-diet interactions: rs758989* dairy intake adjusted for age, sex and BMI (n= 210)

High-fat dairy intake (median: 0.72 portions/d)	G/G		G/A		A/A		P value Genotype	P value High-fat dairy	P value interaction
	Low (n=27)	High (n=35)	Low (n=50)	High (n=46)	Low (n=28)	High (n=24)			
Glucose	4.91±0.49	4.98±0.42	5.05±0.42	4.86±0.45	4.93±0.46	4.93±0.57	0.4147	0.8696	0.2090
Insulin	77.41±42.72	77.88±27.06	82.72±35.36	79.93±36.47	84.59±54.30	103.61±101.38	0.7097	0.2583	0.4639
HOMA-IR	2.37±0.97	2.51±0.95	2.70±1.24	2.49±1.19	2.68±1.73	3.48±4.22	0.3457	0.2341	0.2777

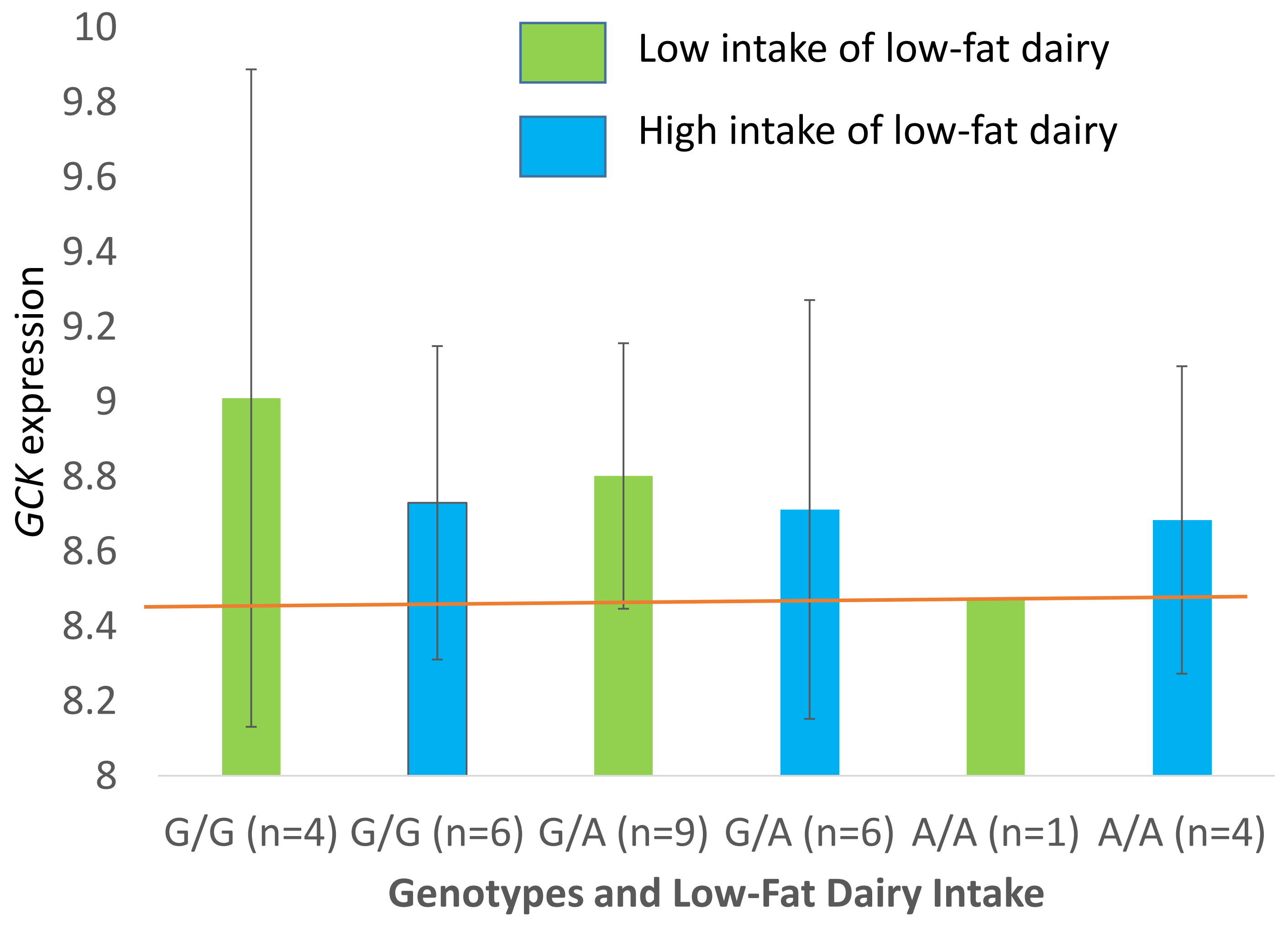
Low-fat dairy intake (median: 1.22 portions/d)	G/G		G/A		A/A		P value Genotype	P value Low-fat dairy	P value interaction
	Low (n=31)	High (n=31)	Low (n=53)	High (n=43)	Low (n=21)	High (n=31)			
Glucose	4.98±0.39	4.92±0.50	4.99±0.43	4.93±0.47	5.09±0.52	4.82±0.48	0.9998	0.3331	0.2321
Insulin	77.37±22.23	77.97±43.7	80.15±41.43	82.91±27.53	121.55±114.55	74.53±32.92	0.7691	0.6573	0.0118
HOMA-IR	2.48±0.75	2.41±1.13	2.58±1.40	2.63±0.95	4.14±4.61	2.32±1.09	0.0880	0.0609	0.0006

Figure 1: HOMA-IR (mean ± SE) according to median low-fat dairy intake and rs758989 genotype adjusted for age, sex and BMI (n= 210)



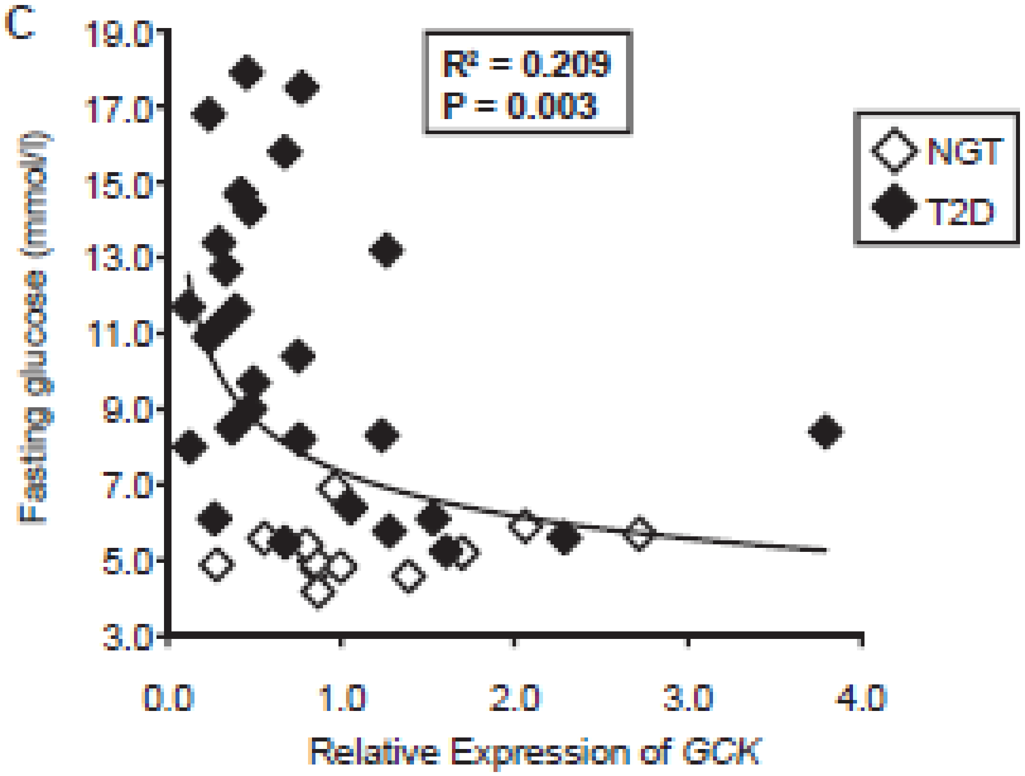
Means with *different letters* are significantly different after a pairwise comparison (Tukey tests; significance <0.05))

Figure 2: GCK gene expression(mean SE) according to median low-fat dairy intake and rs758989 genotype (n=30)



Discussion: GCK polymorphisms

- Common variation in *GCK* predominantly influences **glycolysis** and the rate of **glucose oxidation** in hepatocytes. (Takeuchi et al., 1996)
- Data indicate that common variation in *GCK* with a modest effect on the rate of carbohydrate oxidation contributes to risk of type 2 diabetes.



↓Expression of GCK when HbAc <7.0 mmol/L

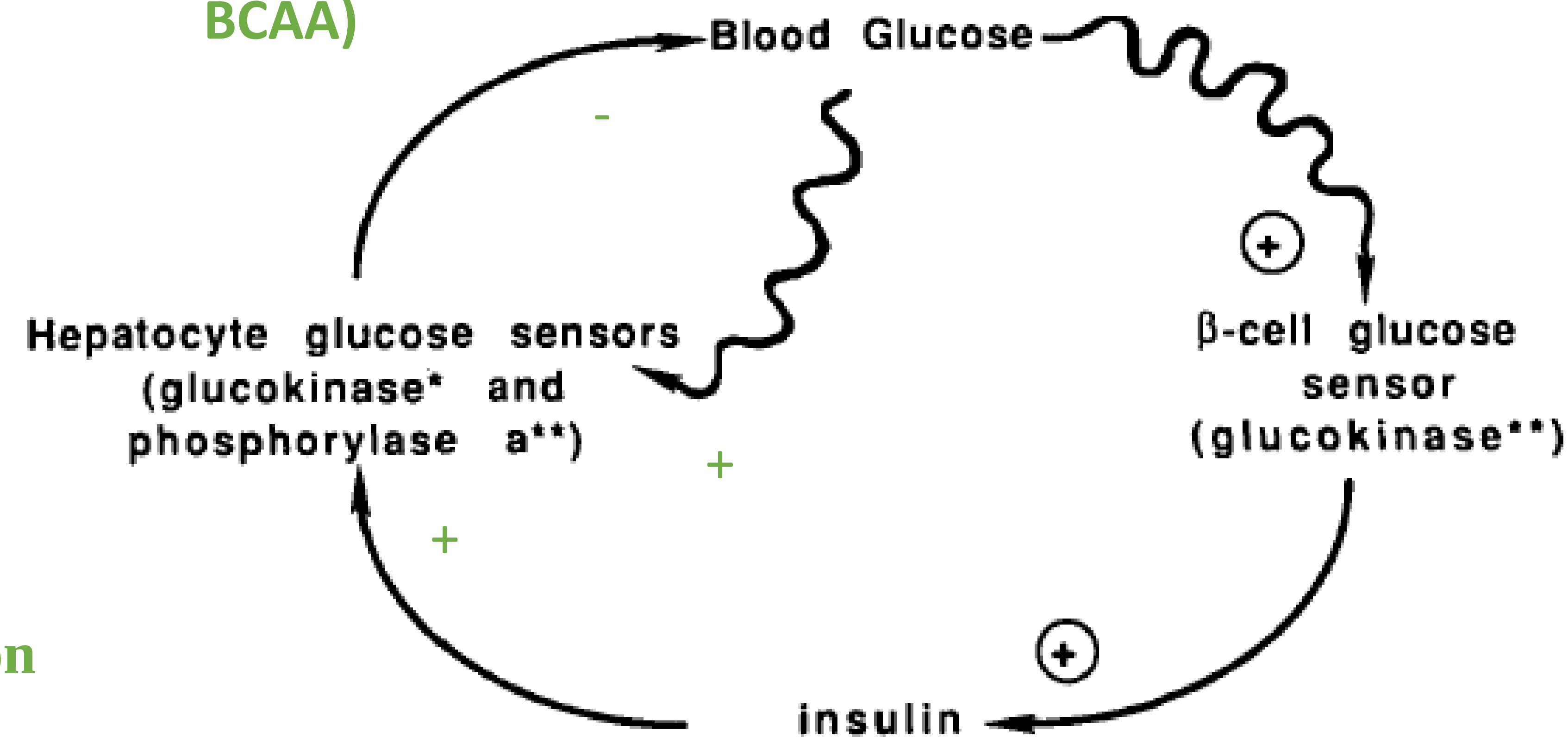
Haeusler RA *et al.*, Mol Met. 2014

GCK and gene expression

Reference	Model	Intervention	GCK expression
Arden et al, 2011	Hepatocytes from male Winstar rats	Low glucose (5 mmol/L)(control) High glucose (25 mmol/L)	↓
Higuchi et al, 2011	HepG2 cells Primary hepatocytes from Winstar rats	Control Branched-Chain Amino Acids (BCAAs)	↑ in both cellular models
Song et al, 2015	INS-1 β-cell line	Glucose 30 mmol/L (control) Curcumin (5–60 μmol/L) Pioglitazone	↑ ↑

Potential mechanism of action: gene-diet interactions

Dairy proteins (Whey protein-BCAA)



Increase GCK gene expression

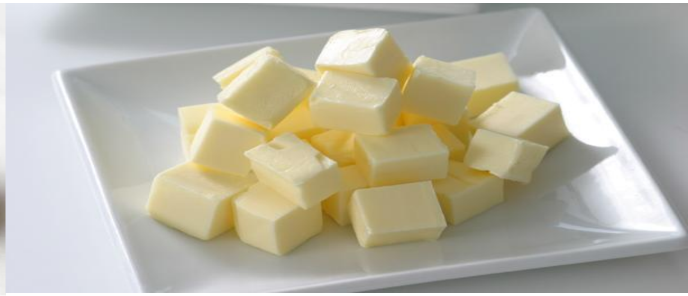
Matschinsky FM, Diabetes 1990

Conclusions

- Overall, intake of LF dairy products may influence glucose-insulin homeostasis in individuals with specific SNPs related to the risk factors of T2D
- Replication studies such as clinical trials with individuals at risk of T2D are needed.
- Future mechanistic studies could confirm gene-diet interactions







Thank you!

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