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REVIEW ARTICLE

Glucose effectiveness: Lessons from studies on insulin-independent glucose clearance in mice

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ABSTRACT

Besides insulin-mediated transport of glucose into the cells, an important role is also played by the non-insulin-mediated transport. This latter process is called glucose effectiveness (acronym S_G), which is estimated by modeling of glucose and insulin data after an intravenous glucose administration, and accounts for ≈70% of glucose disposal. This review summarizes studies on S_G, mainly in humans and rodents with focus on results achieved in model experiments in mice. In humans, S_G is reduced in type 2 diabetes, in obesity, in liver cirrhosis and in some elderly populations. In model experiments in mice, S_G is independent from glucose levels, but increases when insulin secretion is stimulated, such as after administration of the incretin hormones, glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide. S_G is reduced in insulin resistance induced by high-fat feeding and by exogenous administration of glucagon. Glucose-dependent (insulin-independent) glucose disposal is therefore important for glucose elimination, and it is also well regulated. It might be of pathophysiological relevance for the development of type 2 diabetes, in particular during insulin resistance, and might also be a target for glucose-reducing therapy. Measuring S_G is essentially important when carrying out metabolic studies to understand glucose homeostasis.

INTRODUCTION

A major mechanism for glucose disappearance from the circulation is insulin-mediated transport into the cells. However, as shown >80 years ago, there is also a non-insulin-dependent process that is mediated by glucose itself to enhance its uptake and metabolism[1]. This was confirmed >40 years ago, when the minimal modeling of glucose and insulin data from an intravenous glucose tolerance test (IVGTT) showed that non-insulin-mediated processes play a major role in glucose disappearance; these processes were described by the term "glucose effectiveness"[2]. The aim of the present review was to elucidate the relevance of glucose effectiveness for glucose disappearance under various physiological and pathophysiological conditions. Understanding the regulation of glucose effectiveness might also have potential therapeutic benefits for glucose-lowering attempts in type 2 diabetes. We have therefore reviewed the clinical studies reporting glucose effectiveness as estimated from IVGTT, and we have also retrospectively analyzed changes of glucose effectiveness in multiple different conditions in mice, where a series of IVGTTs have been carried out under standardized conditions.

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HISTORY AND DEFINITION

The history of glucose effectiveness goes back to the late 1970s, when Bergman *et al.*[2] formulated the equation system of the minimal model to describe glucose disappearance during an intravenous glucose administration in dogs. They then found that it was not possible to describe glucose disappearance only with the contribution of insulin[2]. Instead, a parameter describing the insulin-independent mechanism was necessarily introduced. This parameter was termed "glucose effectiveness" (p_1), although no specific discussion on p_1 appeared in this first paper, which was focused on insulin sensitivity (S_I). The existence of a non-insulin-dependent glucose disposal was also shown in the first study in humans with the minimal model, where again the parameter p_1 was termed "glucose effectiveness"[3]. Similarly, in a study of glucose uptake in the absence of a sustained insulin response, it was observed that hyperglycemia increases glucose uptake, further suggesting an insulin-independent glucose-dependent glucose uptake in humans[4].

Glucose effectiveness is today referred to as the ability of glucose per se to suppress endogenous glucose production and stimulate peripheral glucose uptake, as was elegantly shown in dogs by Ader *et al.*[5] The acronym for glucose effectiveness that we use today (S_G) was first used in a human study in 1985,

where it was stated that "S_G (formerly p_1) [is] the insulin-independent fractional glucose disappearance"[6]. In the classic review by Bergman *et al.*[7] of the same year that canonized the minimal model approach as a reliable method to assess insulin sensitivity, parameter p_1 was still used in the equations, but it was stated that p_1 is S_G, defined as a "measure of the effect of glucose to enhance its own disappearance [within the extracellular glucose pool] at basal insulin, independent of any increase in insulin". In subsequent years, papers exploiting the minimal model have also reported glucose effectiveness, either as p_1[8,9] or as S_G[10-12], or only mentioned S_G without discussing it[13]. Glucose effectiveness has also been estimated with combined eu- and hyperglycemic clamp[14], with similar conclusions as achieved by the minimal modeling approach, as recently was reviewed[15].

GLUCOSE EFFECTIVENESS AND CLINICAL CONDITIONS

As glucose effectiveness is the "ability of glucose per se without any change in insulin to disappear from blood"[5,16], it quantifies the fractional rate (min^{-1}) of glucose utilization in the brain, central nervous system, red blood cells and other insulin-independent tissues/organs, such as kidneys. Renal excretion of glucose, which is an insulin-independent process, also contributes to S_G. Glucose effectiveness is calculated from a minimal model of insulin and glucose data after an IVGTT[17,18]. The model assumes a first-order non-linear insulin-controlled kinetic, and accounts for the effect of insulin and glucose itself on glucose disappearance after exogenous glucose injection. The model provides two parameters: S_I, which is defined as the ability of insulin to enhance glucose disappearance and inhibit glucose production (i.e., insulin sensitivity), and S_G, representing glucose disappearance from plasma without any change in dynamic insulin[3,17,18]. The mathematical procedure for minimal model parameters (thus S_G) is explained in detail previous studies[17,18], which show how S_G is estimated through a series of mathematical steps when the model differential equations are applied to a set of IVGTT data.

Several early studies documented the large contribution of this insulin-independent glucose disposal to overall glucose disposal in humans[19,20], which was continuously appreciated[21,22], even recently[15,23]. Several studies also examined S_G in various clinical conditions. Table 1 summarizes many of these studies. Studies have thus shown that S_G is reduced in obesity[24], type 2 diabetes[9,25,26], gestational diabetes[27], liver cirrhosis[28] and USA older adults[29,30], whereas S_G is increased in growth hormone deficiency[31] and after administration of glucagon-like peptide-1 (GLP-1)[32-34]. In contrast, S_G is not changed in impaired glucose tolerance[20] or by treatment with thiazolidinediones[35]; the GLP-1 receptor agonist, liraglutide[36]; or the dipeptidyl peptidase-4 (DPP-4) inhibitor, vildagliptin[37], in type 2 diabetes patients; or after carbohydrate dieting in USA older adults[11] or in Italian older adults with a normal oral glucose test[12]. These studies have been undertaken mainly in white people, but the result that S_G is reduced in type 2 diabetes has also been reported in Malaysian[38], Japanese[39] and Chinese people[40], whereas in contrast, similar S_G in type 2 diabetes patients as controls has been reported in African Americans[41] and Ghanaians[42]. Therefore, different ethnic groups might show differences in the impact on type 2 diabetes by S_G. However, in impaired glucose tolerance, S_G was found to be lower than in controls in Japanese people[43], but not reduced in white people[20] or in African Americans[41]. These differences are of interest on the background that type 2 diabetes in Asian people is primarily characterized by impaired insulin secretion rather than an interplay between insulin resistance and failed islet compensation[44]. The finding of reduced S_G in individuals with impaired glucose tolerance among Japanese individuals[43] would suggest that reduced glucose effectiveness contributes to diabetes development in these patients, and this is supported by the results of a study showing reduced S_G in the offspring of Japanese patients with type 2 diabetes even at normal glucose tolerance[45]. However, to study whether the contribution by S_G to the development of type 2 diabetes is different in ethnic groups, direct comparisons need to be carried out in individual studies. One such study has compared S_G in two different ethnic groups (Mexican Americans and non-Hispanic white Americans) showing no difference[46]. However, more studies are required for examining S_G in other ethnic groups.

APPROACH TO STUDY GLUCOSE EFFECTIVENESS IN MICE

To study the physiological and pathophysiological meaning of glucose effectiveness and its mechanism of action, in the 1990s we adapted the minimal model to standardized mouse experiments[18,47]. This allowed more detailed studies on physiology and regulation of S_G, and the knowledge of S_G has therefore been expanded during the past decades. When translated for studies that use the minimal model in mice, the following protocol has been used: after a 5-h fast during the late morning hours, mice (most often from the NMRI or C57BL/6J strain) are anesthetized with an intraperitoneal injection of a fixed-dose combination of fentanyl (0.02 mg/mouse)–fluanisone (0.5 mg/mouse) and midazolam (0.125 mg/ mouse). After 30 min, a blood sample (40 μL) is taken from the retrobulbar, intraorbital sinus capillary plexus in pipette tubes that have been pre-rinsed in heparin solution (100 U/mL in 0.9% NaCl). Thereafter, mice are given an intravenous bolus dose of glucose (dissolved in saline) over a period of 3 s in a tail vein, and whole blood is sampled as aforementioned at 1, 5, 10, 20, 30 and 50 min after glucose injection. Glucose is detected in whole blood, and plasma is immediately separated after collection and stored at −20°C until analysis for insulin. Regarding the possible influence on the estimation of S_G during IVGTT of the renal glucose excretion, it is worth noting that the peak glucose levels after the injection could be above the kidney glucose threshold. However, although it is known that the renal glucose threshold for mice is ≈22 mmol/L[48], such high values are rarely seen after the standard glucose injection or are observed for only a very short period of time after the glucose

Table 1 | Glucose effectiveness in various clinical studies

Studies	Comparisons	S_G (No. participants)	Reference
Obesity	Lean	0.030 ± 0.003 (18)	24
	Obese	0.016 ± 0.002 (18)*	
Type 2 diabetes	Type 2 diabetes	0.014 ± 0.002	25
	Controls	0.024 ± 0.003*	
Type 2 diabetes	Type 2 diabetes	0.016 ± 0.009 (25)	26
	Controls	0.023 ± 0.012 (130)*	
Gestational diabetes	GDM	0.022 ± 0.002 (10)	27
	NGT	0.021 ± 0.003 (9)	
Cirrhosis	Cirrhosis	0.015 ± 0.002 (9)	28
	Controls	0.024 ± 0.003 (6)	
Aging	Mean 65 years	0.017 ± 0.002 (20)	29
	Mean 20 years	0.025 ± 0.002 (20)	
Aging	Young men (aged 18–36 years)	0.029 ± 0.005 (8)	30
	Elderly men (65–82 years)	0.031 ± 0.004 (10)	
GH administration in GH deficiency	Controls	0.020 ± 0.003 (8)	31
	GH deficiency	0.010 ± 0.001 (8) *	
	GH administration in GH deficiency	0.015 ± 0.001 (8) *	
GLP-1 administration in healthy individuals	Controls	0.018 ± 0.001 (6)	32
	GLP-1	0.026 ± 0.003 (6)	
GLP-1 administration in healthy individuals	Controls	0.018 ± 0.002 (17)	33
	GLP-1	0.025 ± 0.002 (17)	
GLP-1 administration in healthy individuals	Controls	0.018 ± 0.002 (10)	34
	$GLP\text{-}1_{7–36}NH_2$	0.025 ± 0.003 (10) *	
	$GLP\text{-}1_{7,37}$	0.024 ± 0.002 (10) *	
	$GLP\text{-}1_{9–36}NH_2$	0.018 ± 0.002 (10)	
Women with IGT	IGT	0.019 ± 0.003 (10)	20
	NGT	0.020 ± 0.003 (10)	
Treatment with TZD of women at high risk for type 2 diabetes	Women with recent GDM and IGT	0.014 ± 0.003 (14)	35
	After 12 weeks TZD treatment	0.015 ± 0.004 (14)	
Treatment with liraglutide in type 2 diabetes	Placebo	Change 0.0008 (–0.003, 0.006)	36
	Liraglutide	Change 0.0016 (–0.0005, 0.006)	
Treatment with vildagliptin in type 2 diabetes	Placebo	0.018 ± 0.002 (14)	37
	Vildagliptin	0.019 ± 0.002 (14)	
Carbohydrate diet	Young men (18–36 years)	0.029 ± 0.005 (8)	11
	Elderly men (65–82 years)	0.027 ± 0.004 (10)	
Type 2 diabetes in Malaysians	Type 2 diabetes	0.012 ± 0.005	38
	Controls	0.025 ± 0.001*	
Type 2 diabetes in Japanese people	Type 2 diabetes	0.011 ± 0.003 (9)	39
	Controls (offspring)	0.024 ± 0.003 (11)*	
Type 2 diabetes in Chinese people	Insulin sensitive type 2 diabetes	0.013 ± 0.008 (71)	40
	Insulin resistant type 2 diabetes	0.016 ± 0.009 (51)*	
Type 2 diabetes and IGT in African Americans	NGT	0.029 ± 0.002 (101)	41
	IGT	0.025 ± 0.002 (36)	
	Type 2 diabetes	0.024 ± 0.002 (17)	
Type 2 diabetes in Ghanaians	Type 2 diabetes	0.023 ± 0.005 (10)	42
	Controls	0.027 ± 0.004 (15)	
IGT in Japanese people	NGT	0.023 ± 0.002 (15)	43
	Insulin-resistant IGT	0.016 ± 0.002 (6)*	
	Insulin sensitive IGT	0.013 ± 0.002 (9)*	
Offspring to Japanese patients with type 2 diabetes	Offspring	0.016 ± 0.003 (10)	45
	Controls	0.023±0.002 (10)*	
Ethnic groups	Mexican Americans	0.022 ± 0.002 (10)	46
	Non-Hispanic whites	0.026 ± 0.008 (11)	

Values are the mean ± standard error or median (95% confidence intervals). *Significant differences between the groups ($P < 0.05$). GDM, gestational diabetes mellitus; GH, growth hormone; GLP-1, glucagon-like peptide-1; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; TZD, thiazolidinedione.

challenge, and therefore, it is likely that the contribution of this process to S_G is negligible.

With this technique, S_G has been shown to be approximately 0.050 min^{-1} in normal mice, with a standard error of the mean of 0.006[47]. This is equivalent to a glucose disposal of 5% of the extracellular glucose pool per min by glucose-dependent insulin-independent mechanisms. This value is higher than the 0.021 min^{-1} reported in humans[22] and 0.028 min^{-1} in dogs[5], but comparable to the reported values in rats, which range from ≈0.040 min^{-1} in obese Zucker rats to ≈0.053 min^{-1} in lean Zucker rats[49], and ≈0.070 min^{-1} in Long-Evans rats[50] and ≈0.090 min^{-1} in endurance-trained animals[51]. Furthermore, several studies have been carried out to understand the factors that might regulate S_G in mice, as it is summarized in Table 2. The incretin hormones, GLP-1 and glucose-dependent insulinotropic polypeptide (GIP), as well as exogenous administration of insulin, increase S_G; whereas S_G is not significantly affected by the neuropeptide, pituitary adenylate cyclase activating polypeptide, or in gastrin-releasing peptide knockout mice, and reduced by glucagon, in GLP-1 receptor knockout mice, in high-fat fed and insulin-resistant mice, as well as after inhibition of insulin secretion[47,52-57]. These data thus show that S_G is a process exposed to a complex regulation, and suggest that S_G might contribute to changes in glucose tolerance under a number of different conditions.

CONTRIBUTION BY GLUCOSE EFFECTIVENESS TO GLUCOSE DISAPPEARANCE

The importance of S_G on glucose tolerance was proposed in a study by Best *et al.*[19] from analyzing the linear regression between intravenous glucose elimination rate, K_G, and S_G. That study showed that insulin-independent glucose uptake contributes by ≈72% to glucose disappearance, indicating that it is the major determinant of intravenous glucose tolerance. This evidence confirmed what was previously shown in dogs[5], where it emerged that S_G contributes by 70–80% to glucose disappearance, later further corroborated by Ader *et al.*[16].

In normal mice, we reached a similar conclusion of a large contribution by glucose effectiveness to glucose disposal with a complex study exploiting IVGTT and glucose clamp[47]. We used sensitivity analysis, which provides estimates of changes of a dependent variable (K_G) for a unit change of independent variables, and accurately describes in quantitative terms the relationships among those variables[47,58]. Some requirements, however, had to be fulfilled for a correct use of this method. First, we showed that S_G is independent from both insulin and S_I in the model. Also, we considered that the total contribution to the net glucose disappearance was ascribed to S_G when insulin did not change. With these assumptions, we showed that insulin (through secretion and effect) contributes to glucose tolerance by 29 ± 6% in normal conditions (Figure 1). Therefore,

Table 2 | Glucose effectiveness in mouse experiments

Studies	Comparisons	S_G (No. animals)	Reference
GIP receptor knockout	GIP receptor knockout	0.061 ± 0.004 (26)	52
	Controls	0.057 ± 0.005 (30)	
GLP-1 receptor knockout	GLP-1 receptor knockout	0.027 ± 0.004 (17)*	52
	Controls	0.044 ± 0.005 (17)	
Incretin hormones	GIP	0.072 ± 0.004 (40)*	53
	GLP-1	0.066 ± 0.005 (47)*	
	Controls	0.045 ± 0.003 (106)	
GRP receptor knockout	GRP receptor knockout	0.052 ± 0.007 (50)	54
	Controls	0.038 ± 0.004 (50)	
High-fat feeding	High-fat feeding for 10 months	0.030 ± 0.004 (24)*	55
	Controls	0.056 ± 0.006 (23)	
Effect of insulin	Insulin administration	0.075 ± 0.004 (48)*	47
	Blocking of insulin secretion	0.014 ± 0.002 (24)*	
	Controls	0.050 ± 0.002 (202)	
PACAP-27	PACAP-27	0.041 ± 0.005 (16)	56
	Controls	0.040 ± 0.006 (16)	
PACAP-38	PACAP-38	0.057 ± 0.008 (24)	56
	Controls	0.043 ± 0.006 (24)	
Glucagon	Glucagon (10 nmol/kg)	0.038 ± 0.004 (24)	57
	Controls	0.058 ± 0.005 (135)	
GLP-1	GLP-1 (3.0 nmol/kg)	0.066 ± 0.005 (47)*	53
	Controls	0.045 ± 0.003 (106)	
GIP	GIP (3.0 nmol/kg)	0.072 ± 0.004 (40)*	53
	Controls	0.045 ± 0.003 (106)	

*Significant differences between the groups ($P < 0.05$). GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; GRP, gastrin releasing peptide; PACAP, pituitary adenylate cyclase activating polypeptide.

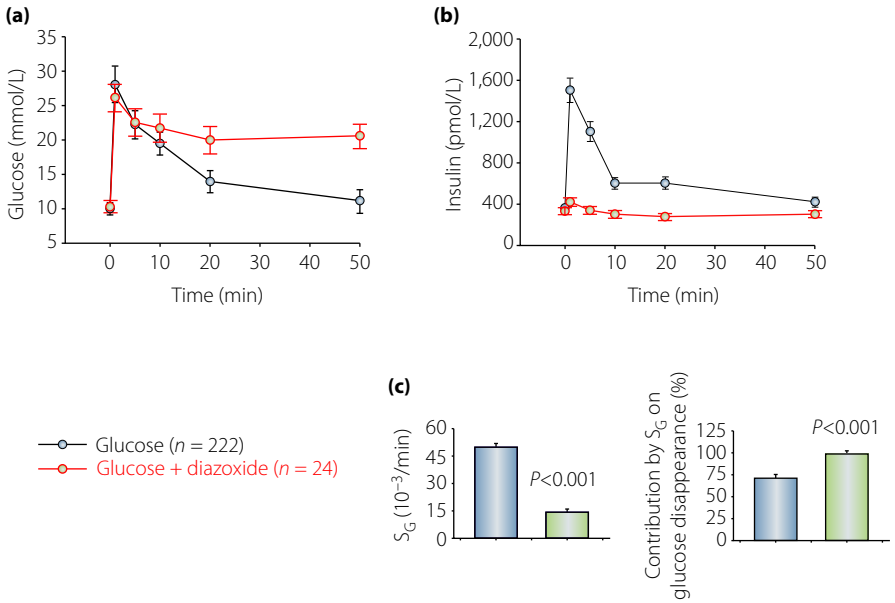

Figure 1 | (a,b) Glucose and insulin concentrations before and after intravenous injection of glucose (1 g/kg) with or without diazoxide (25 mg/kg) in NMRI mice. (c) Glucose effectiveness (S_G) and the relative contribution by S_G on glucose disappearance in the two groups. The mean ± standard error of the mean is shown for glucose and insulin data, and for S_G, and the mean ± standard deviation for the contribution. Data from experiments reported in Pacini *et al.*[47] S_G, glucose effectiveness.

we confirmed that insulin-independent mechanisms; that is, S_G, contributes by more than two-thirds to glucose disappearance.

We also studied S_G when insulin secretion had been completely blocked and therefore no change in dynamic insulin is possible. This was achieved by the drug, diazoxide, which completely inhibits insulin secretion through a direct effect on the β-cells[59]; in mice, it was not possible to use somatostatin, as it never completely inhibited insulin secretion[47]. Diazoxide was administered subcutaneously to mice at the dose of 25 mg/kg 10 min before the intravenous administration of glucose[47]. This resulted in complete inhibition of insulin secretion, but yet an efficient glucose disappearance persisted. Figure 1 shows the results. It is seen that glucose disposal was impaired, but not absent, during diazoxide (in red), although the insulin response was totally inhibited (Figure 1b). S_G contributed by approximately 75% to glucose disposal without diazoxide (Figure 1c). Therefore, elimination of insulin secretion during the intravenous glucose challenge resulted in impairment of glucose disposal by just ≈30%, which verified that insulin-independent mechanisms are quantitatively more important than insulin-dependent mechanisms for glucose disposal[47,58].

RELATIONSHIP BETWEEN GLUCOSE EFFECTIVENESS AND INSULIN

S_G is estimated as the insulin-independent glucose disposal, and should therefore be independent from insulin. However, under certain conditions, there is a relationship between S_G and the insulin secretory function. We verified this by showing that S_G is reduced when insulin secretion is blocked by diazoxide[47]. This could suggest either that S_G is overestimated by the minimal model (as S_G during diazoxide should theoretically estimate the "true" S_G) or that S_G also requires insulin, even though its dynamics are not dependent on changes in insulin. However, when correlating S_G with the area under the insulin curves ($AUC_{insulin}$) in studies with a wide span of insulin concentrations, no correlation was observed, except for extremely elevated values of peak insulin when S_G was reduced, perhaps as a protection against hypoglycemia[47]. These results suggest that basal insulin and S_G synergistically cooperate such that an increase in insulin during IVGTT is required for S_G and, furthermore, that at extremely high insulin levels, S_G is reduced.

RELATIONSHIP BETWEEN GLUCOSE EFFECTIVENESS AND GLUCOSE

To evaluate whether the estimation of S_G is affected by the prevailing glycemia, we collected a series of IVGTT experiments carried out in 83 normal mice (glucose dose 0.35 g/kg)[53]. The total $AUC_{glucose}$ ranged from 380 to 880 mol/L·min in 50 min (averaging 555 ± 11 mol/L·min), and the mean peak (1 min) value of glucose was 17 ± 0.3 mmol/L. The mean S_G was 0.045 ± 0.003 min^{-1}, and did not correlate with either $AUC_{glucose}$ ($R^2 = 0.0003$; $P > 0.5$) or the peak glucose ($R^2 = 10^{-5}$; $P > 0.1$). This shows that the estimation of S_G is independent of glucose levels reached during the tests. This is also evident from a novel ad hoc series of experiments with IVGTT with two different doses of glucose in mice. Mice were anesthetized as explained above, and injected intravenously with glucose at either 0.35 g/kg (low dose; $n = 17$) or at 0.75 g/kg

(high dose; $n = 16$), which yield extremely different glucose levels; samples were taken with the usual protocol, and S_G estimated from glucose and insulin data. The results are reported in Figure 2. It is evident that the estimation of S_G is independent of the glucose levels reached during the test: S_G was 0.053 ± 0.003 min^{-1} at the glucose dose of 0.35 g/kg, and 0.057 ± 0.004 min^{-1} at 0.75 g/kg (not significantly different; $P = 0.47$). Hence, levels of circulating glucose do not affect the assessment of S_G.

RELATIONSHIP BETWEEN GLUCOSE EFFECTIVENESS AND INSULIN RESISTANCE

Elevated insulin is a characteristic of insulin resistance. In humans, insulin resistance in obesity[24], liver cirrhosis[28] and pregnancy with or without gestational diabetes[27] are associated with a 30–50% reduction in S_G. Therefore, it has been of interest to deeply evaluate the role of S_G in insulin resistance; that is, if either S_G follows the pattern of insulin sensitivity or is increased in insulin resistance to augment glucose uptake. To study this, we used mice given a high-fat diet for 10 months[55]. In this model, bodyweight is increased, along with a reduction in insulin sensitivity and an adaptive increase in insulin secretion; nevertheless, glucose disposal is reduced[60]. We carried out IVGTT at 1 week, and 1, 3 and 10 months after initiation of a high-fat diet[55]. As expected, we found that bodyweight increased, S_I was markedly reduced and insulin levels were compensatorily increased. Figure 3 shows the S_G in these experiments. It is seen that S_G was reduced by high-fat feeding, and this effect was already evident after 1 week. The contribution of S_G to glucose disappearance was reduced to approximately 40% at this time point. Interestingly, S_G slightly improved after the first week of high-fat feeding, although it was always lower than in mice fed a control diet. This was at variance with insulin sensitivity, which progressively deteriorated over time in mice fed a high-fat diet. Increased S_G over time in insulin resistance might therefore be a counterbalance of the elevated insulin resistance, but the main conclusion of this study is that insulin resistance is also associated with a reduced S_G, which therefore might add to the glucose intolerance in this condition.

GLUCOSE EFFECTIVENESS AND INCRETIN HORMONES

GLP-1 and GIP are known to stimulate insulin secretion, and therefore enhance insulin levels[61]. This is a major effect behind the development of GLP-1 receptor agonists[62] and DPP-4 inhibitors[63] as glucose-lowering therapy for type 2 diabetes. We carried out a study on the effects of GIP versus GLP-1 in C57BL/6J mice[53]. We found that both incretin hormones augmented glucose-stimulated insulin secretion in a dose-dependent manner[53]. We found that both incretin hormones also increased S_G[53]. Here, we have revisited those data and explored the S_G results in relation to various administered dose of incretin hormone. Interestingly, as seen in Figure 4, GIP was more potent that GLP-1 in augmenting S_G, as a clear effect was observed by the dose of 0.03 nmol/kg, whereas the lowest effective dose of GLP-1 was 10-fold higher. In contrast, an earlier study in NMRI mice showed only modest changes in S_G by

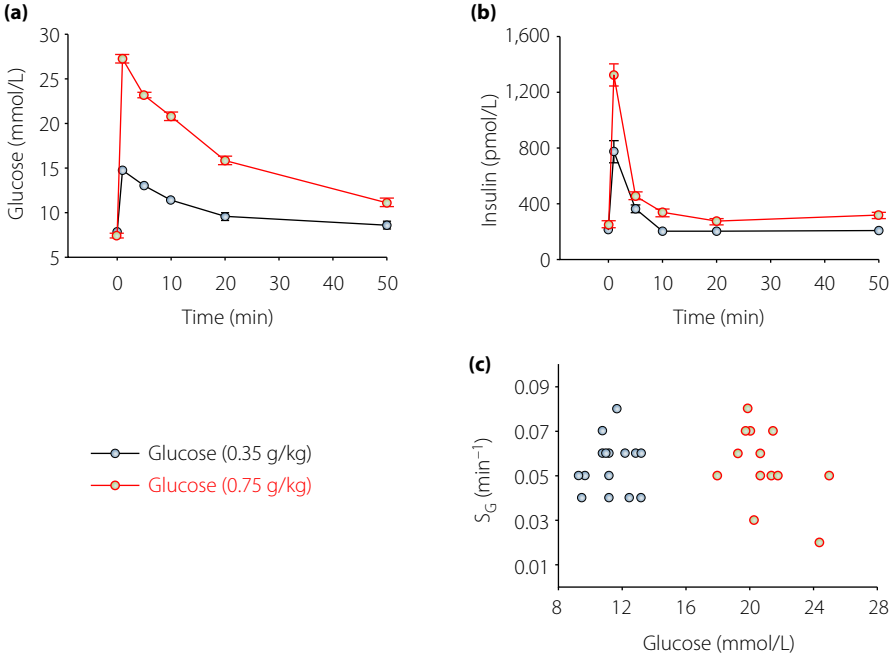

Figure 2(a,b) | Glucose and insulin concentrations before and after intravenous administration of glucose at 0.35 g/kg ($n = 17$) or 0.75 g/kg ($n = 16$) in C57BL/6J mice. The mean ± standard error of the mean is shown. (c) Glucose effectiveness (S_G) versus 1-min glucose level after injection in individual mice in the two groups.

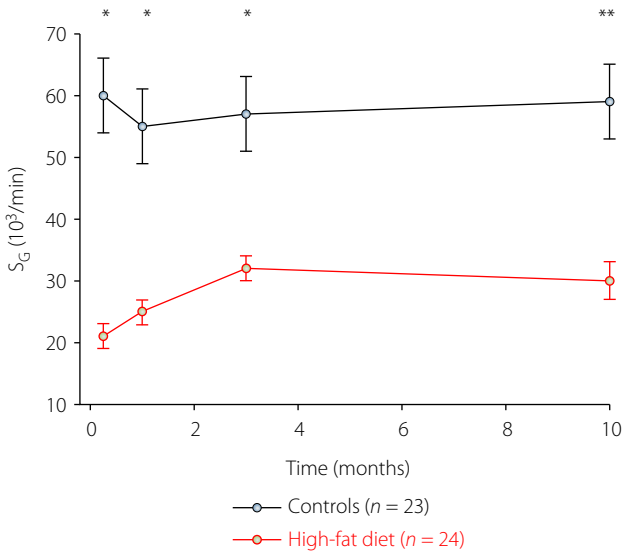

Figure 3 | Glucose effectiveness (S_G) in mice fed a control diet (11% fat; n = 23) or a high-fat diet (58% fat, n = 24) for up to 10 months. The mean ± standard error of the mean is shown. Data from experiments reported by Ahrén *et al.*[55] Asterisks indicate probability level of random difference between the groups, *P < 0.05, **P < 0.01.

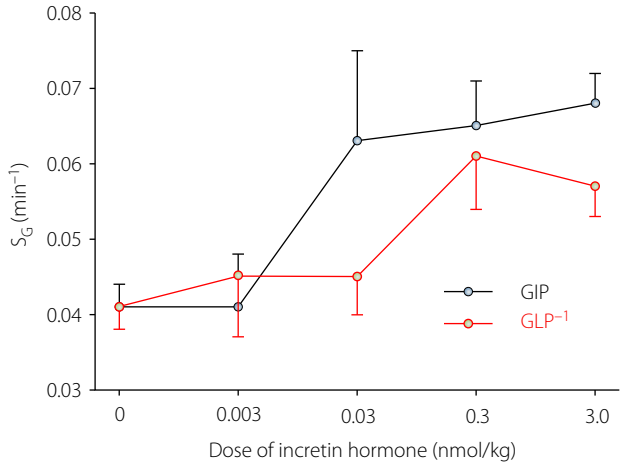

Figure 4 | Glucose effectiveness (S_G) after intravenous administration of glucose-dependent insulinotropic polypeptide (GIP) or glucagon-like peptide-1 (GLP-1) at different dose levels in an intravenous glucose tolerance test in C57BL/6J mice. The mean ± standard error of the mean is shown. There were 83 mice in the glucose-only group (dose 0), and a total of 152 animals in the GLP-1/GIP supplemented groups. Revisited data from results reported by Pacini *et al.*[53]

increasing GLP-1 doses[64]. In humans, it was also shown that GLP-1 augments S_G[32-34]. This suggests that increased S_G, together with the classical incretin effect to stimulate insulin secretion, might be a mechanism to prevent hyperglycemia. This would also be supported by a finding that glucose effectiveness is increased during the early phase of an oral glucose tolerance test when the incretin effect is at its zenith[65]. We have also shown that S_G is reduced in GLP-1 receptor knockout mice, which further shows the impact of GLP-1 on insulin-independent glucose disappearance[52]. In contrast, S_G is not significantly altered in GIP receptor knockout mice[52].

As incretin hormones increase circulating insulin after intravenous glucose, it is still not established whether the increase by GIP and GLP-1 of S_G is due either to the increasing insulin, regardless of the stimulus, or to a primary effect of incretins themselves. Evidence from other studies seem to support the first hypothesis, as other potent enhancers of glucose-stimulated insulin secretion also similarly increase S_G in mice, such as the neuropeptide, pituitary adenylate cyclase activating polypeptide[47,56]. However, as previously discussed, high insulin levels, if anything, reduce S_G; thus, it is more likely that incretin hormones enhance S_G through an extrapancreatic effect independently from their stimulation of insulin secretion. In support of this, we consider again the lack of association between S_G and $AUC_{insulin}$ after GLP-1 and GIP[53]. Such an effect would be consistent with extrapancreatic actions of GIP[66,67]; also, GLP-1 has been shown to have extrapancreatic effects that might directly (through the liver) or indirectly (through neural effects) affect glucose disposal[68-71].

An interesting consequence of the finding of enhanced S_G by incretin hormones is that the proportion of the relative contribution of insulin-dependent and non-insulin-dependent mechanisms to glucose disposal is increased, which was significant for GIP. Thus, a GIP-induced increase in glucose disappearance was associated with a higher dependency on S_G than after glucose alone and after glucose plus GLP-1[53]. This suggests that GIP enhances the processes driving non-insulin-dependent glucose clearance, which, again, would fit with extrapancreatic actions of GIP.

GLP-1 receptor agonists and DPP-4 inhibitors are frequently used as antihyperglycemic therapy in type 2 diabetes patients[61-63]. Both these therapies work through GLP-1 receptors, the GLP-1 receptor agonists by achieving a pharmacological activation of the receptors, and DPP-4 inhibitors by preventing the inactivation of endogenously produced GLP-1, thereby increasing the GLP-1 receptor activation by endogenous GLP-1. It is therefore of interest to discuss whether the improved S_G observed when GLP-1 is administered to healthy volunteers[32-34] might contribute to the metabolic benefits of these therapies. One study explored this by comparing S_G after 12 weeks of treatment with the GLP-1 receptor agonist liraglutide in combination with metformin versus metformin alone in type 2 diabetes for 12 weeks using a cross-over design[36], and another study explored the effect of the DPP-4 inhibitor, vildagliptin, versus a placebo during 10 days of treatment in type 2 diabetes patients[37]. It was found, however, that neither liraglutide nor vildagliptin did increase S_G in these studies[36,37]. This would therefore suggest that although GLP-1 is able to increase S_G in

healthy individuals, therapy with GLP-1 receptor agonists or DPP-4 inhibitors does not seem to increase the low S_G associated with type 2 diabetes. This could be explained by the reduced S_G in type 2 diabetes, which might be more difficult to increase than in healthy individuals, but it might also be due to a failure of GLP-1 to continuously increase S_G over a long period of time. Further studies are required to solve whether GLP-1 receptor agonists and DPP-4 inhibitors affect S_G during prolonged treatment of type 2 diabetes.

GLUCOSE EFFECTIVENESS AND GLUCAGON

The decrease of glucose concentration during the IVGTT after the peak caused by the bolus glucose injection is mainly due to glucose uptake and inhibition of glucose production. It is known that glucagon is strictly related to endogenous (liver) glucose production; therefore, studying the effects of glucagon on S_G could provide information on the probable actions that this pancreatic hormone exerts on glucose effectiveness and, consequently, hypothesize possible relationships between S_G and glucose production.

To this aim, glucagon at different doses was added to the glucose bolus[57]. The results show (Table 1) that supplementing glucagon to glucose reduces S_G by approximately 30% on average[57]. This indicates that glucagon diminishes glucose effectiveness, suggesting that S_G reflects glucose production during hyperglycemia. As GLP-1 increases S_G, we carried out a series of experiments in mice where GLP-1 was added to glucagon. This addition, however, did not modify S_G compared with glucagon alone, indicating that GLP-1 does not increase S_G under conditions when glucagon levels are elevated. We conclude that glucagon is more potent as an inhibitor of S_G than GLP-1 as an enhancer.

POSSIBLE MECHANISMS OF GLUCOSE EFFECTIVENESS

Glucose per se is a fundamental substrate for liver metabolism[72], and understanding the mechanisms of its regulation is paramount. Glucose effectiveness plays an essential role in this regulation; however, the molecular mechanisms underlying glucose effectiveness are not well defined yet. A study in individuals with hepatic cirrhosis showed that S_G is reduced by 38%, which explained 65% of the glucose intolerance in these individuals[28]. This would be consistent with a hypothesis that S_G is exerted in the liver, where S_G would be linked to the stimulation of glucose uptake. However, as liver cells do not have the capacity to take up glucose, and there is no correlation between S_G and liver enzymes in cirrhotic patients[28], it is more likely that the reduction of S_G in cirrhotic patients is a result of a reduced muscle mass, suggesting that S_G is primarily exerted in the muscles[73]. Glucose transporters might be candidates for new studies; for instance, it is known that the glucose transporter 4 causes entry of glucose into muscular cells after its translocation to the membrane[74]. However, as the molecular bases for S_G are still largely unknown, further studies on these topics are required.

RELEVANCE OF MONITORING INSULIN-INDEPENDENT GLUCOSE DISPOSAL

As already seen, S_G has been evaluated in several clinical conditions (Table 1), where it has been shown to vary, making it a relevant factor for the assessment of glucose tolerance and turnover of an individual. It is worth noting that the recent availability of sodium–glucose cotransporter 2 inhibitors as antidiabetic agents has offered a therapeutic approach acting directly on the kidneys without requiring insulin action[75,76]. In line with this, sodium–glucose cotransporter 2 inhibition has been shown to improve the reduced glucose effectiveness in the liver in diabetic Zucker fatty rats[77]. For this reason, glucose effectiveness might become a fundamental parameter for the evaluation of the influence of such compounds on glucose disposal. When the molecular mechanisms underlying S_G are more established, there will also be a potential to target these mechanisms to increase S_G in glucose-lowering therapy of type 2 diabetes patients.

CONCLUSIONS

Glucose effectiveness describes the processes of insulin-independent mechanisms of glucose disposal. It is estimated by modeling glucose and insulin data after an intravenous glucose administration, and it accounts for ≈70% of glucose disposal. It is reduced in type 2 diabetes[9] and obesity[24,78], and experimental model studies in mice have characterized the regulation of glucose effectiveness with special emphases on the role of glucose, insulin and processes stimulating insulin secretion. It is essential, therefore, to evaluate this parameter any time a metabolic test is carried out, especially in large population studies[79]. Further studies are warranted to explore the regulation of glucose effectiveness, its molecular basis and the potential of targeting glucose effectiveness as a glucose-lowering approach in type 2 diabetes patients.

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DISCLOSURE

The authors declare no conflict of interest.

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