Research Paper

Experimental Physiology

Enhanced insulin secretion and glucose tolerance in rats exhibiting low plasma free fatty acid levels and hypertriglyceridaemia due to congenital albumin deficiency

Tiago R. Figueira[1]*, Rosane A. Ribeiro[2]*, Letícia M. Ignacio-Souza[2], Anibal E. Vercesi[1], Everardo M. Carneiro[2] and Helena C. F. Oliveira[2]

[1] *Departamento de Patologia Clínica, Faculdade de Ciências Médicas, Universidade Estadual de Campinas, Campinas, SP, Brazil*
[2] *Departamento de Fisiologia e Biofísica, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, SP, Brazil*

Congenitally analbuminaemic individuals and rats (NARs) exhibit several metabolic abnormalities, including hypertriglyceridaemia and plasma free fatty acid deficiency. Our aim was to study glucose homeostasis and insulin secretion in NARs. Plasma concentrations of lipids, glucose and insulin and secretion of insulin from the pancreatic islets were measured in female NARs and control animals (Sprague–Dawley rats; SDRs). Glucose homeostasis tests were also performed. Plasma glucose levels were similar between NARs and SDRs, irrespective of feeding status. However, fed insulinaemia was ∼37% higher ($P \leq 0.05$) in NARs than in SDRs. The NARs displayed a markedly increased glucose tolerance, i.e. the integrated glycaemic response was one-third that of the control animals. Enhanced glucose tolerance was associated with threefold higher insulinaemia at peak glycaemia after a glucose load than in the control animals. Similar peripheral insulin sensitivity was observed between groups. Isolated pancreatic islets from NARs secreted significantly more insulin than islets from SDRs in response to a wide range of glucose concentrations (2.8–33.3 mM). Despite having similar liver glycogen contents in the fully fed state, NARs had ∼40% ($P \leq 0.05$) lower glycogen contents than SDRs after 6 h fasting. The injection of a gluconeogenic substrate, pyruvate, elicited a faster rise in glycaemia in NARs compared with SDRs. Overall, NARs displayed enhanced glucose tolerance, insulin secretion and gluconeogenic flux. The higher glucose tolerance in NARs compared with SDRs is attributed to enhanced islet responsiveness to secretagogues, while peripheral insulin sensitivity seems not to be involved in this alteration. We propose that the enhanced glucose metabolism is a chronic compensatory adaptation to decreased free fatty acid availability in NARs.

(Received 3 October 2011; accepted after revision 22 December 2011; first published online 23 December 2011)
Corresponding author H. C. F. Oliveira: Departamento de Fisiologia e Biofísica, Instituto de Biologia, Universidade Estadual de Campinas, Rua Monteiro Lobato, 255, Campinas – SP, Brazil, CEP 13083-862. Email: ho98@unicamp.br

Congenital analbuminaemia is a rare autosomal recessive disorder characterized by very low levels of plasma albumin ($<1\ \mathrm{mg\ ml^{-1}}$) in the absence of renal and intestinal protein loss (Weinstock *et al.* 1979). This abnormality results from negligible hepatic albumin production due to mutations in the albumin gene (Minchiotti *et al.* 2008). The first case of human analbuminaemia was reported in 1954 (Kallee, 1996), and several additional cases of human analbuminaemia have been identified. By selectively breeding spontaneously hyperlipidaemic Sprague–Dawley rats (SDRs), Nagase *et al.* (1979) established a colony of rats, Nagase analbuminaemic rats (NARs), that were confirmed to be analbuminaemic. These rats model many features of human familial analbuminaemia (Baldo-Enzi *et al.* 1987).

* T.R.F. and R.A.R. contributed equally to this work.

© 2012 The Authors. Experimental Physiology © 2012 The Physiological Society
DOI: 10.1113/expphysiol.2011.062307
Downloaded from Exp Physiol (ep.physoc.org) at CAPES - Usage on June 11, 2014

The most remarkable metabolic alterations in congenital analbuminaemic humans and rats are high levels of plasma lipids and lipoproteins (Kikuchi *et al.* 1983; Joles *et al.* 1993; Catanozzi *et al.* 1994; Newstead *et al.* 2004), but a myriad of comorbidities have been described in cases of human analbuminaemia and in NARs (Kallee, 1996; Koot *et al.* 2004; Newstead *et al.* 2004; Neuhaus *et al.* 2008; Figueira *et al.* 2011). Many endocrine and metabolic alterations have been shown in NARs, including hypothyroidism, hyperparathyroidism, low adiposity and body mass, and plasma free fatty acid (FFA) deficiency (Ando *et al.* 1980; Kikuchi *et al.* 1983; Takahashi *et al.* 1984; Yamamoto *et al.* 1992; Inaba *et al.* 2000). Despite these abnormalities, NARs thrive in standard laboratory housing conditions.

Kawaguchi *et al.* (1986) showed that NARs are severely intolerant (i.e. early mortality) to food deprivation at 5°C ambient temperature compared with control SDRs. Interestingly, these authors found that the survival time was greatly extended when NARs were fed a carbohydrate-rich diet, while a fat-rich diet did not extend survival. It is well known that NARs have a plasma FFA deficiency (Ando *et al.* 1980). Recent data from our group indicate that the NAR plasma FFA deficiency arises from diminished intravascular lipolysis owing to the lack of albumin to act as a FFA acceptor (Figueira *et al.* 2010). Importantly, nearly 80% of lipolysis-derived FFAs are mixed within the pool of albumin-bound FFAs before their uptake into peripheral tissues (Teusink *et al.* 2003; Voshol *et al.* 2009); therefore, the absence of plasma albumin and the FFA deficiency in NARs is expected to reduce FFA flux into tissues. The low NAR adiposity (Kikuchi *et al.* 1983) and carbohydrate-dependent intolerance to starvation (Kawaguchi *et al.* 1986) are in accordance with a low FFA availability and flux into tissues (Voshol *et al.* 2009). Taken together, these studies suggest that NAR energy metabolism relies more heavily on carbohydrates than on fats compared with control SDRs.

Pancreatic insulin secretion and the action of insulin on peripheral tissues are affected by circulating FFA levels (Randle *et al.* 1963; Frayn, 2003; Haber *et al.* 2003). Chronic high levels of plasma FFAs may lead to glucose intolerance owing to the impairment of both glucose-stimulated insulin secretion (GSIS) and insulin sensitivity in target tissues (Randle *et al.* 1963; Mason *et al.* 1999). Conversely, acute increases in plasma FFA levels enhance GSIS (Dobbins *et al.* 1998; Carpentier *et al.* 1999), whereas an impaired GSIS is observed when plasma FFA levels are acutely lowered by pharmacological methods (Stein *et al.* 1996; Dobbins *et al.* 1998). The effects of chronically low levels of plasma FFA on insulin secretion and action have never been investigated. Hypothetically, according to Randle's cycle, chronically low plasma FFA availability might facilitate glucose metabolism in NAR tissues (Randle *et al.* 1963; Frayn, 2003). The influence of chronically low plasma FFA levels on GSIS seems less predictable.

From this framework, our general hypothesis was that NARs display enhanced carbohydrate metabolism. Indeed, in the present study, we demonstrate that NARs deplete more liver glycogen stores during fasting and have a higher glucose tolerance than SDRs. The increased glucose tolerance is associated with enhanced GSIS, while there is no difference in peripheral insulin sensitivity between NARs and control SDRs.

Methods

Ethical approval

Experiments were approved by the local university Committee for Ethics in Animal Experimentation, which is under the guidance of the Brazilian Society for Science in Laboratory Animals (Sociedade Brasileira de Ciências de Animais de Laboratório).

Materials

^{125}I-labelled human insulin was purchased from Genesis (São Paulo, SP, Brazil). Unless otherwise stated, all other reagents were of the highest grade available from Sigma (St Louis, MO, USA).

Animal housing and plasma variable assessments

Nagase analbuminaemic rat founders were kindly donated by Dr Eder Quintão from the Lipid Laboratory at the University of São Paulo Medical School and were bred and maintained in our departmental animal facility. Control (Sprague–Dawley) rats were obtained from the university breeding colony. Female rats, 12–14 weeks old, were housed at 22 ± 2°C with a 12 h–12 h light–dark cycle. Body masses (mean $\pm$ SEM) were 286 ± 8 and 216 ± 4 g, respectively, for SDRs and NARs. The SDR and NAR groups were age matched. The rats had free access to standard laboratory rodent chow diet (Nuvital CR1, Parana, Brazil) and water. The measurements of experimental variables were carried out in the fed state and/or after 20 h of fasting. Blood samples were taken from the tip of tail for the determination of triglycerides (Roche Diagnostics, Mannheim, Germany), total cholesterol (Roche Diagnostics, Mannheim, Germany) and free fatty acids (Wako chemicals, Osaka, Japan) in plasma using colorimetric enzymatic methods according to the manufacturer's instructions. Plasma insulin levels were measured by radioimmunoassay as previously described (Scott *et al.* 1981), and blood glucose was measured with a portable analyser (Accu-Chek Advantage; Roche, Mannheim, Germany). Animals were killed by

© 2012 The Authors. Experimental Physiology © 2012 The Physiological Society
Downloaded from Exp Physiol (ep.physoc.org) at CAPES - Usage on June 11, 2014

decapitation at the end of the study or before organ harvesting.

Glucose tolerance test (GTT)

Fasted rats were injected intraperitoneally with glucose (2 g (kg body weight)$^{-1}$). Blood samples were taken from the tip of the tail to determine blood glucose before (0 min) and after the glucose injection (30, 60 and 120 min). The glucose response to GTT was calculated as the area under the glucose curve for each rat (Microcal Origin 8.0, Northampton, MA, USA). Another set of animals was used to measure plasma insulin and glucose levels at various time points (0, 15 and 60 min) during the GTT.

Insulin tolerance test

Fasted rats were injected intraperitoneally (1.5 U (kg body weight)$^{-1}$) with human recombinant insulin (Biohulin®; Biobrás, São Paulo, Brazil). Blood samples were taken from the tip of the tail before (0 min) and after the insulin injection (4, 8, 12, 16 and 20 min) for blood glucose analysis.

Islet isolation, static insulin secretion and islet insulin content

Pancreatic islets were isolated by collagenase digestion of the pancreas as previously described (Scott *et al.* 1981; Ribeiro *et al.* 2010). For static incubations, four islets from each group were first incubated for 30 min at 37°C in Krebs–bicarbonate buffer of the following composition (mM): 115 NaCl, 5 KCl, 2.56 $CaCl_2$, 1 $MgCl_2$, 10 $NaHCO_3$ and 15 Hepes, supplemented with 5.6 mM glucose and 3 g l^{-1} bovine serum albumin, and equilibrated with a mixture of 95% O_2–5% CO_2 to give a pH of 7.4. The medium was then replaced with fresh buffer and the islets were incubated for 1 h with crescent glucose concentrations (2.8, 5.6, 8.3, 11.1, 16.7 or 33.3 mM glucose) or with 2.8 mM glucose without or with 10 mM L-leucine, 15 mM α-ketoisocaproic acid (KIC) or 30 mM KCl. At the end of the incubation period, the insulin content of the medium was measured by radioimmunoassay. For islet insulin content, groups of four islets were collected and transferred to 1.5 ml tubes. One millilitre of deionized water was added to these samples, followed by sonication of the pancreatic cells (three times, 10 s pulses), and the islet insulin content was measured by radioimmunoassay.

Effects of acute correction of NAR plasma FFA deficiency on glucose tolerance

In another study conducted in parallel (Figueira *et al.* 2010), we recently showed that intravascular albumin injection into NARs elicits an elevation in NAR plasma FFA levels over time. A complete abrogation of NAR plasma FFA deficiency was observed 90 min postalbumin injection. To investigate whether the acute correction of plasma FFA deficiency rescues the high glucose tolerance in NARs, these rats were injected with albumin (1.3 g kg^{-1}, intravenously) and the intraperitoneal GTT was performed, as described above, 90 min later. Both SDR and NAR control groups were injected with an equivalent volume of saline (2.7 ml kg^{-1}).

The response of liver glycogen content to short-term fasting

The livers were harvested after decapitation of fed and fasted rats. The fed rats had full access to food and were killed 2 h after feeding. Other groups of fasted rats were killed 6 or 14 h following food withdrawal. Glycogen was measured by the phenolsulfuric method (Lo *et al.* 1970) in liver samples (pieces weighing 15–20 mg from the two major lobules) after KOH digestion and ethanol precipitation of glycogen. The content of liver glycogen was calculated against a standard curve built with D-glucose.

Glycaemic response to pyruvate injection

The increase in blood glucose after pyruvate injection is dependent on gluconeogenesis flux (Miyake *et al.* 2002). Rats fasted for 14 h were injected intraperitoneally with pyruvate (2 g (kg body weight)$^{-1}$). A 0.333 g ml^{-1} stock solution of sodium pyruvate salt (Merck, Darmstadt, Germany) was prepared in saline immediately prior to use. Blood glucose was measured before and after the injection (15, 30, 60, 90, 120 and 180 min). The time to reach the peak glycaemic value after pyruvate injection was taken as an index of glucose augmentation rate in the blood.

Statistical analysis

Data are presented as means $\pm$ SEM. Differences among means were assessed by unpaired Student's *t* test, one-way or two-way ANOVA, followed by the Newman–Keuls *post hoc* test. The Mann–Whitney *U* test was used to analyze non-parametric data shown in Figure 5*B*. When multiple unpaired Student's *t* tests were conducted, the *P* value was corrected according to Cross & Chaffin (1982). Correlation was assessed by the Person coefficient. The significance level was set at $P \leq 0.05$.

Results

The plasma levels of lipids, glucose and insulin from fasted and fed rats are reported in Table 1. The NARs show higher plasma levels of triglycerides (approximately fourfold)

© 2012 The Authors. Experimental Physiology © 2012 The Physiological Society

Downloaded from Exp Physiol (ep.physoc.org) at CAPES - Usage on June 11, 2014

Table 1. Plasma biochemical variables in Nagase analbuminaemic (NARs) and control rats (SDRs)

Variable	State	SDR	NAR
Glucose (mg dl^{-1})	Fasting	70.4 ± 3.5	69.7 ± 2.3
	Fed	97.7 ± 2.5	98.4 ± 3.8
Insulin (ng ml^{-1})	Fasting	0.24 ± 0.06	0.29 ± 0.06
	Fed	0.95 ± 0.11	1.3 ± 0.12*
Triglycerides (mg dl^{-1})	Fasting	78.5 ± 11.7	406.9 ± 34.3*
	Fed	59.9 ± 4.8	269.7 ± 46*
Cholesterol (mg dl^{-1})	Fasting	67.5 ± 5.8	215.7 ± 22.0*
	Fed	58.3 ± 6.8	191.4 ± 11.8*
Free fatty acids (mM)	Fasting	0.94 ± 0.1	0.35 ± 0.03*
	Fed	0.6 ± 0.04	0.11 ± 0.01*

Values are means ± SEM. *$P \leq 0.05$ *versus* SDR.

and cholesterol (approximately threefold) than SDRs, irrespective of feeding state, while FFA levels were lower in both fed (∼80%) and fasted NARs (∼66%) compared with SDRs. Plasma glucose did not differ between NARs and SDRs. Insulinaemia was ∼37% higher in fed NARs than in fed SDRs ($P \leq 0.05$), but there was no difference between these groups in the fasted state (Table 1).

An intraperitoneal GTT revealed a markedly higher ($P \leq 0.05$) glucose tolerance in NARs than in SDRs (Fig. 1*A*), as indicated by a nearly 60% reduction in the area under the glycaemic curve (Fig. 1*B*). The insulinaemic response during GTT was also assessed at the 0, 15 and 60 min time points. The NARs presented higher ($P \leq 0.05$) insulinaemia at 15 (threefold) and 60 min (twofold) after the glucose load (Fig. 1*C*). The peripheral insulin sensitivity, as assessed by glycaemic decay after the intraperitoneal injection of insulin (insulin tolerance test), did not differ between NARs and SDRs (Fig. 1*D*). These data suggest that the higher glucose tolerance in NARs compared with SDRs seems to be caused by enhanced pancreatic insulin secretion. Therefore, we measured GSIS rates from pancreatic islets isolated from both groups of rats. Figure 2*A* shows the pattern of static insulin secretion

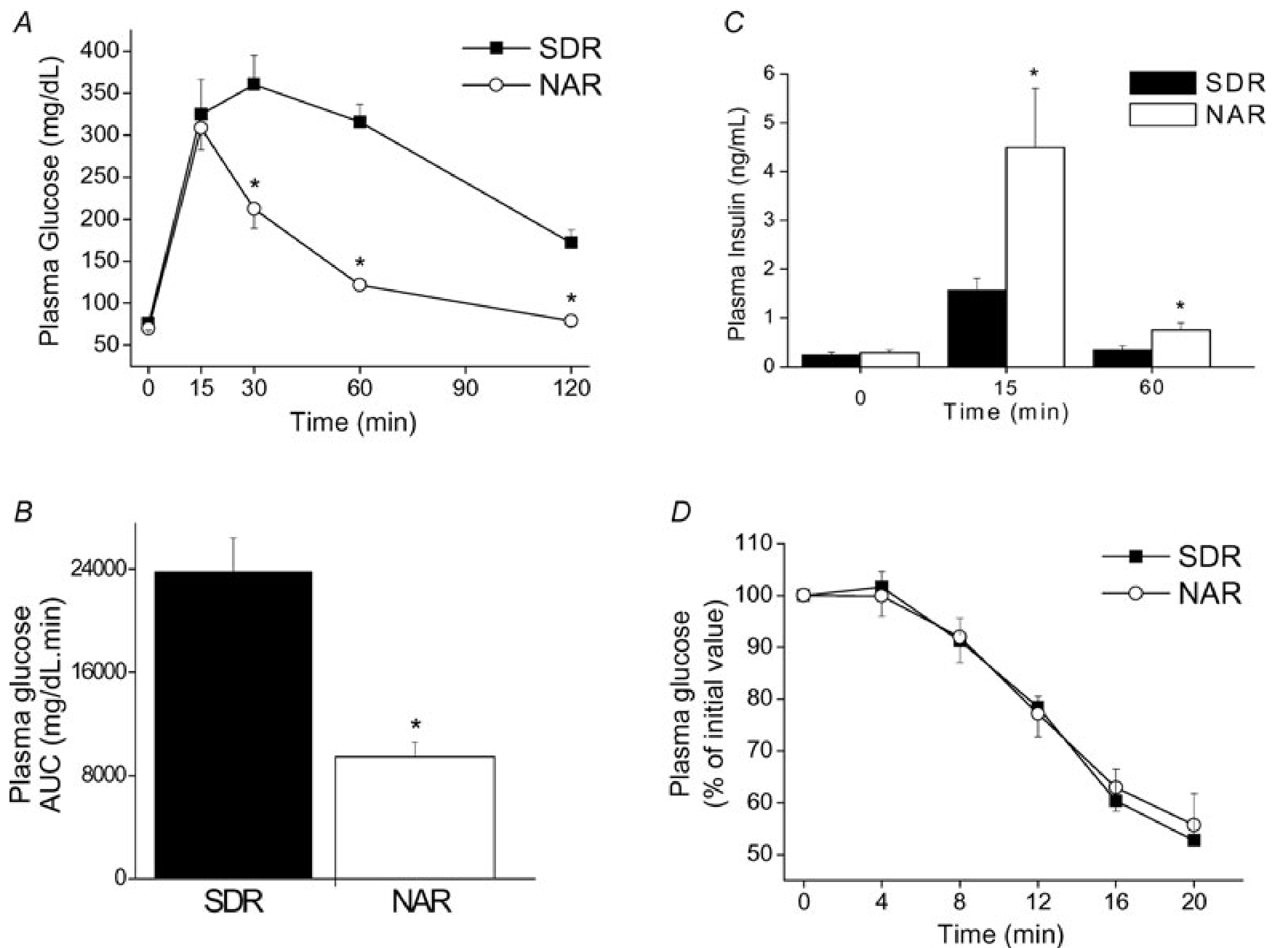

Figure 1. Enhanced glucose tolerance and insulin response to a glucose load but similar peripheral insulin sensitivity in Nagase analbuminaemic rats (NARs) compared with Sprague–Dawley rats (SDRs)
Values are means ± SEM. *A*, blood glucose responses to the intraperitoneal glucose tolerance test (GTT). *B*, areas under the curve (AUCs) for blood glucose during the GTT. *C*, plasma insulin levels during the GTT. *D*, blood glucose response to the insulin tolerance test. *$P \leq 0.05$ *versus* SDR.

© 2012 The Authors. Experimental Physiology © 2012 The Physiological Society
Downloaded from Exp Physiol (ep.physoc.org) at CAPES - Usage on June 11, 2014

in response to increasing concentrations of glucose (from 2.8 to 33.3 mM). The NARs demonstrated increased insulin secretion in response to all concentrations of glucose tested ($P \leq 0.05$). These *in vitro* data are in agreement with the enhanced insulin response to the glucose load observed *in vivo* (Fig. 1*C*). The enhanced NAR GSIS is not due to any alteration in insulin synthesis or storage, because islet insulin content was similar between the groups (40.4 ± 4.2 and 39.5 ± 5.4 ng per islet in SDRs and NARs, respectively).

In addition, we also evaluated the effect of other insulin secretagogues, such as L-leucine, KIC or KCl, as direct depolarizing stimuli (Fig. 2*B*). Leucine is known to stimulate insulin secretion by the following two mechanisms: first, via leucine catabolism and, second, via allosteric activation of glutamate dehydrogenase (GDH), which promotes an increase in the formation of α-ketoglutarate from glutamate (i.e. anaplerosis; Sener & Malaisse, 1980; Kelly *et al.* 2002). We found higher insulin secretion from NAR islets ($P \leq 0.05$) upon leucine stimulation (Fig. 2*B*). However, KIC, a product of leucine catabolism that does not possess the GDH stimulatory action, elicited similar stimulation of insulin secretion in NAR and SDR islets (Fig. 2*B*). Thus, the higher NAR islet responsiveness to leucine may be due to enhanced GDH activity. In addition, the similar insulin secretion between NAR and SDR islets in response to KIC suggests a normal β-cell mitochondrial metabolism in NARs. Enhanced insulin secretion was also observed in NAR islets ($P \leq 0.05$) in response to direct membrane depolarization by KCl (Fig. 2*B*), a stimulus that bypasses islet nutrient metabolism.

It is noteworthy that the levels of plasma insulin were inversely correlated ($r = -0.68$, $P \leq 0.05$) with the plasma levels of FFAs when data from fed NARs and SDRs were analysed together (Fig. 3). In an attempt to examine the influence of FFA on glucose homeostasis directly, a GTT was performed after acute correction of plasma FFA deficiency by exogenous administration of albumin to NARs. The acute abrogation of NAR plasma FFA deficiency induced by albumin injection as demonstrated by Figueira *et al.* (2010) did not affect the enhanced glucose tolerance in NARs (areas under the curve were 22,222 ± 765, 15,003 ± 197 and 15,090 ± 1214 mg dl^{-1} min, respectively, for saline-injected SDRs, saline-injected NARs and albumin-injected NARs, respectively), suggesting that chronic modifications in glucose metabolism and/or insulin secretion machinery were promoted by the long-term low-FFA environment.

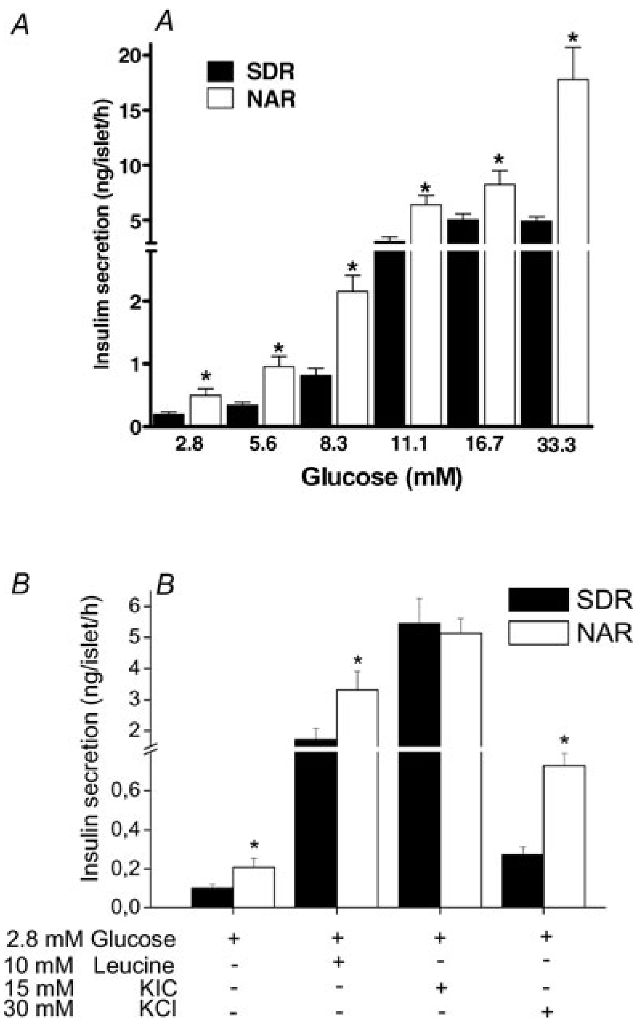

Figure 2. Enhanced insulin secretion from isolated NAR pancreatic islets
A, insulin secretion rate in response to increasing glucose concentration. *B*, insulin secretion rates in response to L-leucine, α-ketoisocaproate (KIC) and KCl. *$P \leq 0.05$ *versus* SDR.

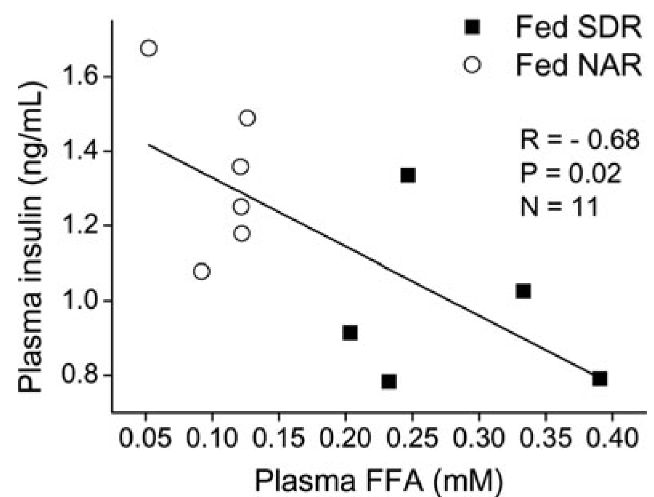

Figure 3. Correlation between the levels of plasma free fatty acids (FFA) and insulin
Data from fed SDR and NAR groups were pooled together and analysed with Pearson's correlation test.

© 2012 The Authors. Experimental Physiology © 2012 The Physiological Society
Downloaded from Exp Physiol (ep.physoc.org) at CAPES - Usage on June 11, 2014

Given that NARs are more tolerant to glucose and secrete more insulin than SDRs (Figs 1 and 2), it is expected that substrate metabolism in these animals relies more on carbohydrates than on fat stores. Thus, liver glycogen depletion in response to short-term fasting was evaluated (Fig. 4). Liver glycogen content did not differ between NARs and SDRs in the fully fed state. Six hours of fasting induced a nearly 40% depletion in NAR liver glycogen stores ($P \le 0.05$), while the SDR liver glycogen content remained unchanged at this time point. Fasting for 14 h led to a marked depletion in both NARs and SDRs and similar liver glycogen content in NARs and SDRs. These data raise the hypothesis that gluconeogenesis may also be enhanced, thereby allowing NARs to cope with greater carbohydrate utilization. Indeed, injection of the gluconeogenic precursor, pyruvate, into fasted rats elicited a faster increase in blood glucose over time in NARs than in SDRs (Fig. 5*A*). Peak glycaemia was attained earlier in NARs (30 min) than in SDRs (∼120 min; Fig. 5*B*), indicating a faster gluconeogenesis rate in the former. The faster decay of glycaemia after the peak value observed in NARs may be a consequence of enhanced islet insulin responsiveness to high blood glucose (Fig. 1*C*).

Discussion

In this work, we showed that NAR, displayed improved glucose tolerance as a consequence of enhanced GSIS (Figs 1*C* and 2*A*). The enhanced GSIS from isolated NAR pancreatic islets was elicited by both metabolic and depolarizing stimuli (Fig. 2*B*). Increased *in vivo* fed insulinaemia was correlated inversely with FFA plasma levels (Fig. 3). Peripheral insulin sensitivity was not altered with high plasma triglyceride and low FFA availability (Fig. 1*D*).

Glucose intolerance associated with metabolic diseases can be improved by interventions such as weight loss, exercise or pharmacotherapy (Matthaei *et al.* 2000), but very few conditions/interventions are associated with improved glucose tolerance above that found in healthy individuals. An example is exercise-trained individuals, who exhibit enhanced glucose tolerance above normal individuals (Wirth *et al.* 1981). Generally, the interventions that improve glucose tolerance, in health or in disease, do so mainly by increasing insulin sensitivity in target tissues, with no involvement or mild involvement of pancreatic insulin secretion (Matthaei *et al.* 2000). Another important point is that only rare metabolic states, such as insulinoma (Marks & Samols, 1968), hypothalamic obesity syndrome (Lustig, 2002) and congenital hyperinsulinaemia (Otonkoski *et al.* 2003), present enhanced islet insulin secretion above the peripheral tissue needs. These are unhealthy, abnormal states associated with enhanced insulin secretion in response to glucose or other stimuli (e.g. glucagon,

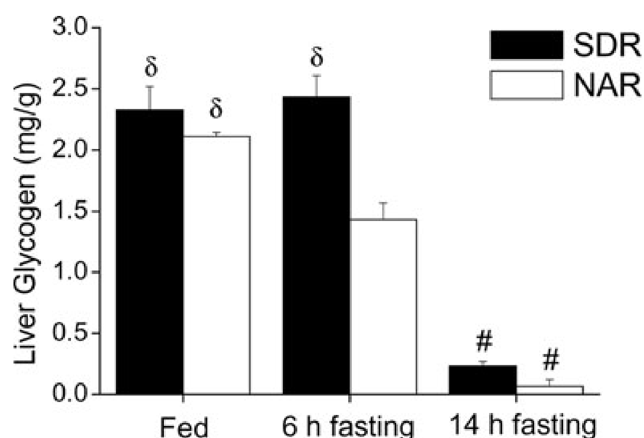

Figure 4. Faster liver glycogen depletion in response to short-term fasting in NARs
Liver glycogen content was measured in different sets of rats in the fully fed state and after 6 or 14 h fasting. Bars not sharing the same symbol are different from each other ($P \le 0.05$).

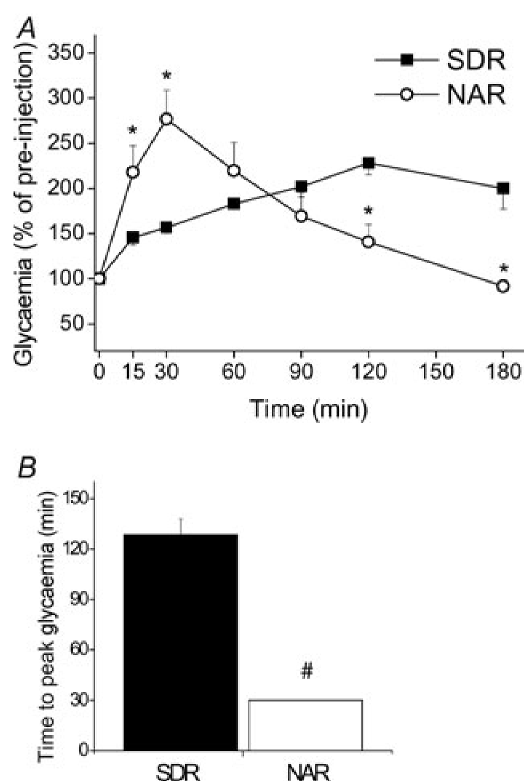

Figure 5. Blood glucose response to intraperitoneal injection of the gluconeogenic precursor, pyruvate (2 g kg^{-1})
A, mean ± SEM of glycaemia expressed as a percentage of the pre-injection value. *B*, mean ± SEM of the time to achieve peak glycaemia after pyruvate injection. *$P \le 0.05$ *versus* SDR at the same time point (panel *A*). #$P < 0.05$ versus SDR (the Mann Whitney *U* test was used for data in panel *B*)..

© 2012 The Authors. Experimental Physiology © 2012 The Physiological Society

Downloaded from Exp Physiol (ep.physoc.org) at CAPES - Usage on June 11, 2014

pyruvate), often resulting in hypoglycaemic crisis (Marks & Samols, 1968; Otonkoski *et al.* 2003). The enhanced insulin secretion in NARs may be the first evidence of a positive compensatory adaptation of pancreatic islets to cope with a whole-body oxidative fuel limitation. Low plasma FFA availability in NARs could shift substrate partitioning towards carbohydrates (Randle *et al.* 1963). Indeed, our results show greater NAR liver glycogen depletion (Fig. 4) and enhanced gluconeogenic flux (Fig. 5) in response to fasting, relative to control SDRs. These data support the hypothesis that NAR energy metabolism relies more on carbohydrates than on FFAs. In addition, a previous study showed that carbohydrate, but not fat, feeding can extend the survival of NARs maintained in a cold environment (Kawaguchi *et al.* 1986); therefore, the enhanced GSIS seems to be functionally important for NAR fuel metabolism.

Chronically high levels of plasma FFAs may cause insulin resistance and impair GSIS (Randle *et al.* 1963; Mason *et al.* 1999). Acute lowering of plasma FFAs is experimentally feasible and also impairs insulin secretion (Stein *et al.* 1996; Dobbins *et al.* 1998; Haber *et al.* 2003), but models to study the effects of chronically low FFAs are lacking. Plasma FFA deficiency in analbuminaemia has long been recognized (Ando *et al.* 1980; Baldo-Enzi *et al.* 1987). In this work, the acute correction of NAR plasma FFA deficiency did not affect glucose tolerance. It is important to highlight that the adaptations for enhanced insulin secretion in NARs persist in the isolated islets functioning in the *ex vivo* conditions (Fig. 2). These findings suggest that NARs have developed long-term adaptations in glucose metabolism and in insulin secretory machinery that are not acutely reversible. Thus, the direct investigation into the role of plasma FFA deficiency on enhanced glucose tolerance and insulin secretion would require a chronic correction of NAR plasma FFA deficit. Nonetheless, an inverse statistical correlation (Fig. 3) between plasma FFA levels and insulinaemia strengthens the hypothesis that decreased plasma FFA levels may play a key role in enhanced insulin secretion in NARs.

In theory, other factors could also contribute to the higher insulin secretion, such as differences in body weight and increased availability of fat fuel to pancreatic islets due to hypertriglyceridaemia. It is known that overweight animals generally present higher insulin secretion in order to cope with increased body mass tissues or with eventual peripheral insulin resistance. However, in the present case, the NARs were lighter than the SDRs, although both groups were within the normal range. Regarding hypertriglyceridaemia, it is very unlikely that high plasma triglycerides are powering the enhanced insulin secretion in NARs because higher GSIS is observed in *in vitro* conditions devoid of lipid substrates and because plasma triglyceride hydrolysis and tissue uptake of lipolysis-derived FFA are hampered in NARs owing to the lack of albumin as an FFA acceptor (Shearer *et al.* 2000; Teusink *et al.* 2003; Figueira *et al.* 2010). Overall, NARs seem to present an inability to use fat as an energy source (Kawaguchi *et al.* 1986).

Hypertriglyceridaemia is generally associated with insulin resistance and type II diabetes (Berthezene, 1992). In general, high plasma levels of triglycerides increase FFA availability to tissues as a result of their peripheral hydrolysis by lipoprotein lipase. Overexpression of lipoprotein lipase leads to FFA oversupply to tissues and causes cellular lipotoxicity and insulin resistance (Ferreira *et al.* 2001). Thus, the relationship between hypertriglyceridaemia and glucose intolerance may arise because most hypertriglyceridaemias are generally accompanied by high levels of plasma FFAs. The unique NAR lipidaemic phenotype, i.e. simultaneous hypertriglyceridaemia and FFA deficiency, indicates that hypertriglyceridaemia *per se* does not negatively affect glucose tolerance or insulin sensitivity. We conclude that analbuminaemic rats exhibit enhanced carbohydrate metabolism, namely higher glycogen disposal, gluconeogenic flux and glucose tolerance, when compared with control rats. The enhanced glucose tolerance is a consequence of enhanced insulin secretion in response to glucose and does not involve abnormal peripheral insulin sensitivity. The molecular mechanisms responsible for enhanced insulin secretion are not known and deserve further investigation.

References

Ando S, Kon K, Tanaka Y, Nagase S & Nagai Y (1980). Characterization of hyperlipidemia in Nagase analbuminemia rat (NAR). *J Biochem* **87**, 1859–1862.

Baldo-Enzi G, Baiocchi MR, Vigna G, Andrian C, Mosconi C & Fellin R (1987). Analbuminaemia: a natural model of metabolic compensatory systems. *J Inherit Metab Dis* **10**, 317–329.

Berthezene F (1992). Hypertriglyceridemia: cause or consequence of insulin resistance? *Horm Res* **38**, 39–40.

Carpentier A, Mittelman SD, Lamarche B, Bergman RN, Giacca A & Lewis GF (1999). Acute enhancement of insulin secretion by FFA in humans is lost with prolonged FFA elevation. *Am J Physiol Endocrinol Metab* **276**, E1055–E1066.

Catanozzi S, Rocha JC, Nakandakare ER, Oliveira HC & Quintao EC (1994). Nagase analbuminemic rats have faster plasma triacylglycerol and VLDL synthesis rates. *Biochim Biophys Acta* **1212**, 103–108.

Cross EM & Chaffin WW (1982). Use of the binomial theorem in interpreting results of multiple tests of significance. *Educational and Psychological Measurement* **42**, 25–34.

Dobbins RL, Chester MW, Stevenson BE, Daniels MB, Stein DT & McGarry JD (1998). A fatty acid-dependent step is critically important for both glucose- and non-glucose-stimulated insulin secretion. *J Clin Invest* **101**, 2370–2376.

© 2012 The Authors. Experimental Physiology © 2012 The Physiological Society

Downloaded from Exp Physiol (ep.physoc.org) at CAPES - Usage on June 11, 2014

Ferreira LD, Pulawa LK, Jensen DR & Eckel RH (2001). Overexpressing human lipoprotein lipase in mouse skeletal muscle is associated with insulin resistance. *Diabetes* **50**, 1064–1068.

Figueira TR, Castilho RF, Saito A, Oliveira HC & Vercesi AE (2011). The higher susceptibility of congenital analbuminemic rats to Ca^{2+}-induced mitochondrial permeability transition is associated with the increased expression of cyclophilin D and nitrosothiol depletion. *Mol Genet Metab* **104**, 521–528.

Figueira TR, Vercesi AE & Oliveira HC (2010). Lack of plasma albumin impairs intravascular lipolysis and explains the associated free fatty acids deficiency and hypertriglyceridemia. *Lipids Health Dis* **9**, 146.

Frayn KN (2003). The glucose-fatty acid cycle: a physiological perspective. *Biochem Soc Trans* **31**, 1115–1119.

Haber EP, Ximenes HM, Procópio J, Carvalho CR, Curi R & Carpinelli AR (2003). Pleiotropic effects of fatty acids on pancreatic β-cells. *J Cell Physiol* **194**, 1–12.

Inaba M, Morii H, Katsumata T, Goto H, Ishimura E, Kawagishi T, Kamao M, Okano T & Nishizawa Y (2000). Hyperparathyroidism is augmented by ovariectomy in Nagase analbuminemic rats. *J Nutr* **130**, 1543–1547.

Joles JA, Feingold KR, Van Tol A, Cohen LH, Sun X, Jones H Jr, Davies RW & Kaysen GA (1993). Extrahepatic lipogenesis contributes to hyperlipidemia in the analbuminemic rat. *Am J Physiol Renal Physiol* **265**, F70–F76.

Kallee E (1996). Bennhold's analbuminemia: a follow-up study of the first two cases (1953–1992). *J Lab Clin Med* **127**, 470–480.

Kawaguchi T, Shimode M, Matsushita H & Nagase S (1986). Frequent administration of uric acid extends survival of fasting analbuminemic rats under cold environment. *Jpn J Physiol* **36**, 295–303.

Kelly A, Li C, Gao Z, Stanley CA & Matschinsky FM (2002). Glutaminolysis and insulin secretion: from bedside to bench and back. *Diabetes* **51**(Suppl 3), S421–S426.

Kikuchi H, Tamura S, Nagase S & Tsuiki S (1983). Hypertriacylglycerolemia and adipose tissue lipoprotein lipase activity in the Nagase analbuminemic rat. *Biochim Biophys Acta* **744**, 165–170.

Koot BG, Houwen R, Pot DJ & Nauta J (2004). Congenital analbuminaemia: biochemical and clinical implications. A case report and literature review. *Eur J Pediatr* **163**, 664–670.

Lo S, Russell JC & Taylor AW (1970). Determination of glycogen in small tissue samples. *J Appl Physiol* **28**, 234–236.

Lustig RH (2002). Hypothalamic obesity: the sixth cranial endocrinopathy. *Endocrinologist* **12,** 210–217.

Marks V & Samols E (1968). Glucagon test for insulinoma: a chemical study in 25 cases. *J Clin Pathol* **21**, 346–352.

Mason TM, Goh T, Tchipashvili V, Sandhu H, Gupta N, Lewis GF & Giacca A (1999). Prolonged elevation of plasma free fatty acids desensitizes the insulin secretory response to glucose in vivo in rats. *Diabetes* **48**, 524–530.

Matthaei S, Stumvoll M, Kellerer M & Haring HU (2000). Pathophysiology and pharmacological treatment of insulin resistance. *Endocr Rev* **21**, 585–618.

Minchiotti L, Galliano M, Kragh-Hansen U & Peters T Jr (2008). Mutations and polymorphisms of the gene of the major human blood protein, serum albumin. *Hum Mutat* **29**, 1007–1016.

Miyake K, Ogawa W, Matsumoto M, Nakamura T, Sakaue H & Kasuga M (2002). Hyperinsulinemia, glucose intolerance, and dyslipidemia induced by acute inhibition of phosphoinositide 3-kinase signaling in the liver. *J Clin Invest* **110**, 1483–1491.

Nagase S, Shimamune K & Shumiya S (1979). Albumin-deficient rat mutant. *Science* **205**, 590–591.

Neuhaus TJ, Stallmach T & Genewein A (2008). A boy with congenital analbuminemia and steroid-sensitive idiopathic nephrotic syndrome: an experiment of nature. *Eur J Pediatr* **167**, 1073–1077.

Newstead J, Card SE & Lyon AW (2004). Low serum albumin and abnormal body shape in a young Canadian first nations woman. *Laboratory Medicine* **35**, 350–356.

Otonkoski T, Kaminen N, Ustinov J, Lapatto R, Meissner T, Mayatepek E, Kere J & Sipila I (2003). Physical exercise-induced hyperinsulinemic hypoglycemia is an autosomal-dominant trait characterized by abnormal pyruvate-induced insulin release. *Diabetes* **52**, 199–204.

Randle PJ, Garland PB, Hales CN & Newsholme EA (1963). The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet* **1**(7285), 785–789.

Ribeiro RA, Vanzela EC, Oliveira CA, Bonfleur ML, Boschero AC & Carneiro EM (2010). Taurine supplementation: involvement of cholinergic/phospholipase C and protein kinase A pathways in potentiation of insulin secretion and Ca^{2+} handling in mouse pancreatic islets. *Br J Nutr* **104**, 1148–1155.

Scott AM, Atwater I & Rojas E (1981). A method for the simultaneous measurement of insulin release and B cell membrane potential in single mouse islets of Langerhans. *Diabetologia* **21**, 470–475.

Sener A & Malaisse WJ (1980). L-Leucine and a nonmetabolized analogue activate pancreatic islet glutamate dehydrogenase. *Nature* **288**, 187–189.

Shearer GC, Joles JA, Jones H, Walzem RL & Kaysen GA (2000). Estrogen effects on triglyceride metabolism in analbuminemic rats. *Kidney Int* **57**, 2268–2274.

Stein DT, Esser V, Stevenson BE, Lane KE, Whiteside JH, Daniels MB, Chen S & McGarry JD (1996). Essentiality of circulating fatty acids for glucose-stimulated insulin secretion in the fasted rat. *J Clin Invest* **97**, 2728–2735.

Takahashi M, Wakabayashi K & Nagase S (1984). Hormone levels of anterior pituitary gland and serum in Nagase analbuminemia rats. *Endocrinol Jpn* **31**, 185–193.

Teusink B, Voshol PJ, Dahlmans VE, Rensen PC, Pijl H, Romijn JA & Havekes LM (2003). Contribution of fatty acids released from lipolysis of plasma triglycerides to total plasma fatty acid flux and tissue-specific fatty acid uptake. *Diabetes* **52**, 614–620.

Voshol PJ, Rensen PC, van Dijk KW, Romijn JA & Havekes LM (2009). Effect of plasma triglyceride metabolism on lipid storage in adipose tissue: studies using genetically engineered mouse models. *Biochim Biophys Acta* **1791**, 479–485.

© 2012 The Authors. Experimental Physiology © 2012 The Physiological Society

Downloaded from Exp Physiol (ep.physoc.org) at CAPES - Usage on June 11, 2014

Weinstock JV, Kawanishi H & Sisson J (1979). Morphologic, biochemical and physiologic alterations in a case of idiopathic hypoalbuminemia (analbuminemia). *Am J Med* **67**, 132–139.

Wirth A, Diehm C, Mayer H, Mörl H, Vogel I, Björntorp P & Schlierf G (1981). Plasma C-peptide and insulin in trained and untrained subjects. *J Appl Physiol* **50**, 71–77.

Yamamoto Y, Wakabayashi K, Niki E & Nagao M (1992). Comparison of plasma levels of lipid hydroperoxides and antioxidants in hyperlipidemic Nagase analbuminemic rats, Sprague-Dawley rats, and humans. *Biochem Biophys Res Commun* **189**, 518–523.

Acknowledgements

This work was supported by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Instituto Nacional de Obesidade e Diabetes (INCT). T.R.F. is a PhD student currently supported by a FAPESP fellowship. We are grateful to Sergio Catanozzi from the University of São Paulo Medical School, who provided mating pairs of Nagase rats, and to Roberto C. Stahl for his technical assistance.

Author's present address

R. A. Ribeiro: Núcleo em Ecologia e Desenvolvimento Sócio-ambiental de Macaé (NUPEM), Universidade Federal do Rio de Janeiro (UFRJ), Macaé, RJ, Brazil.

© 2012 The Authors. Experimental Physiology © 2012 The Physiological Society

Downloaded from Exp Physiol (ep.physoc.org) at CAPES - Usage on June 11, 2014