Insulin Causes \([\text{Ca}^{2+}]_i\)-Dependent and \([\text{Ca}^{2+}]_i\)-Independent Positive Inotropic Effects in Failing Human Myocardium

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Background—Insulin has been shown to exert positive inotropic effects in several in vitro and in vivo models, but signal transduction and substrate dependency remain unclear. We examined inotropic responses and signal transduction mechanisms of insulin in human myocardium.

Methods and Results—Experiments were performed in isolated trabeculae from end-stage failing hearts of 58 nondiabetic and 3 diabetic patients undergoing heart transplantation. The effect of insulin (0.3 and 3 IU/L) on isometric twitch force (37°C, 1 Hz) was tested in the presence of glucose or pyruvate as energetic substrate. Furthermore, intracellular \(\text{Ca}^{2+}\) transients (aequorin method), sarcoplasmatic reticulum (SR) \(\text{Ca}^{2+}\) content (rapid cooling contractures), and myofilament \(\text{Ca}^{2+}\) sensitivity (semiskinned fibers) were assessed. In addition, potential signaling pathways were tested by blocking glycolysis, PI-3-kinase, protein kinase C, diacylglycerol kinase, insulin-like growth factor-1 receptors, or transsarcolemmal \(\text{Ca}^{2+}\) entry via the \(\text{Na}^+/\text{Ca}^{2+}\) exchanger. Insulin exerted concentration-dependent and partially substrate-dependent positive inotropic effects. The phosphatidylinositol-3-kinase inhibitor wortmannin and the \(\text{Na}^+/\text{Ca}^{2+}\) exchanger reverse-mode inhibitor KB-R7943 completely or partially prevented the functional effects of insulin. In contrast, insulin-like growth factor-1 receptor blockade, protein kinase C inhibition, and diacylglycerol kinase blockade were without effect. The inotropic response was associated with increases in intracellular \(\text{Ca}^{2+}\) transients, SR \(\text{Ca}^{2+}\) content, and increased myofilament \(\text{Ca}^{2+}\) sensitivity.

Conclusions—Insulin exerts \(\text{Ca}^{2+}\)-dependent and \(\text{Ca}^{2+}\)-independent positive inotropic effects through a phosphatidylinositol-3-kinase-dependent pathway in failing human myocardium. The increased \([\text{Ca}^{2+}]_i\), originates at least in part from enhanced reverse-mode \(\text{Na}^+/\text{Ca}^{2+}\) exchange and consequently increased SR-Ca\(^{2+}\) load. These nongenomic functional effects of insulin may be of clinical relevance, eg, during insulin-glucose-potassium infusions. (Circulation. 2005;111:2588-2595.)

Key Words: insulin ■ calcium ■ heart failure ■ myocardium ■ contractility

Patients with chronic heart disease often have insulin resistance, diabetes, or altered glucose metabolism.1 Insulin, a polypeptide of 51 amino acids, is known to regulate serum glucose levels, protein synthesis, and growth. Effects of insulin on myocardial function have been tested in several animal models under in vitro and in vivo conditions, and controversial results with both enhanced contractility and myocardial performance2–6 or no functional effects7,8 have been reported. Insulin infusions improved ventricular function in one study in humans9 but had no effect in another.10 In addition, the functional response to insulin may be different in diabetic versus nondiabetic animals3,11,12 and humans.9,13

Recently, the onset of insulin resistance has been shown to coincide with progression from pressure-overload hypertrophy to dilatation.14 Whole-body insulin resistance is prevalent in congestive heart failure patients with either ischemic heart failure or idiopathic dilated cardiomyopathy.15,16 Therefore, insulin resistance may contribute to contractile dysfunction by genomic, metabolic, and direct functional effects. Despite the controversial reports on direct effects of insulin on myocardial function in mammalian myocardium, no data are available that directly test the inotropic response to insulin in human myocardium. In addition, the mechanisms of action of the functional effect of insulin in mammalian myocardium remains controversial.5 Under in vivo conditions, improved global ventricular function during insulin infusions9 might exclusively result from insulin-dependent arteriolar vasodilation with peripheral unloading13,17 and improved myocardial blood flow.17 Therefore, we directly assessed functional effects and mechanisms of action of insulin in isolated failing human myocardium. Our main findings were that insulin exerts both \(\text{Ca}^{2+}\)-dependent and \(\text{Ca}^{2+}\)-independent positive inotropic effects. These functional effects were related to activation of phosphatidylinositol-3-kinase (PI-3-kinase) and, in part, reverse-mode \(\text{Na}^+/\text{Ca}^{2+}\) exchange. These data help to improve our understanding of nongenomic hormonal effects in the human heart.
Methods

Human Myocardium

Experiments were performed in muscle strips obtained from 58 end-stage failing hearts (23 of ischemic origin and 35 of dilative origin; 50 males and 8 females, all without diabetes). Three additional hearts were obtained from patients with long-standing diabetes mellitus. The mean age of the patients was 51.8 ± 2.3 years, and the mean ejection fraction before transplantation was 23.7 ± 1.2%. The study protocol was approved by the local ethics committee, and all patients gave informed consent.

Muscle Strip Preparation

Small endocardial trabeculae were dissected from the left or right ventricle as described previously,18 connected to an isometric force transducer, and superfused with bicarbonate-containing Tyrode’s solution. Muscles were electrically stimulated at 1 Hz (37°C), and isometric contractions were recorded at optimum preload (Lmax). The functional effects of insulin were assessed with 2 different concentrations (0.3 and 3 IU/L) and 2 different substrate conditions of the Tyrode solution (either 11.2 mmol/L glucose or 22.4 mmol/L pyruvate).

Aequorin Measurements

At steady state contractile function, the Ca2+-regulated bioluminescent photoprotein aequorin was microinjected into the quiescent muscle. Aequorin light emission was detected with a photomultiplier, which was vertically mounted with its cathode just above the glass cuvette that contained the muscle.

Rapid Cooling Contractures

Rapid cooling contractures (RCCs) were elicited by a rapid decrease in the temperature of the muscle chamber from 37°C to 1°C by switching from a warm to a cold solution with solenoid pinch valves at the bath inlet as previously described.19 The resulting cooling contracture is an index for sarcoplasmic reticulum (SR) Ca2+ content.

Semiskinned Fiber Preparations

Fibers were skinned by the technique and solutions described by Hambarchian et al.20 Saponin (50 μg/mL) was added for 45 minutes to functionally skin the trabeculae. This technique allows the control of intracellular ion concentrations but maintains sarcolemmal receptors and leaves subcellular signaling mechanisms intact.21 Concentration-response curves for Ca2+ (tension-Ca2+ relationship) were obtained in each muscle, first without insulin and after complete reequilibration in the presence of 3 IU/L insulin.

Drugs

Insulin (Insuman Rapid 100 IU/mL, Hoechst Marion Russel) was used as supplied. Insulin-like growth factor-1 (IGF-1) receptor antibody (α-IR-3 clone; Oncogene Research Products) was dissolved as recommended by the manufacturer. KB-R 7943 (5 μmol/L; Tocris), diacylglycerol (DAG)-kinase inhibitor (1 μmol/L), ryanodine (10 μmol/L), GF109203 (1 μmol/L), cyclopiazonic acid (10 mmol/L), ryanodine (1 μmol/L), wortmannin (0.1 μmol/L), and iodoacetate (0.5 μmol/L; all from Sigma) were added to the organ bath 30 minutes before the experiment.

Statistical Analysis

Data are expressed as mean ± SEM. Differences were compared by paired Student t test or 1-way repeated-measures ANOVA followed by Student-Newman-Keuls test when appropriate. Statistical significance was taken as P < 0.05.

Results

Inotropic Effects of Insulin

Insulin exerted pronounced transient positive inotropic effects in isolated human cardiac muscle (Figure 1A) without changes in diastolic tension. These functional effects of insulin were concentration dependent in glucose-containing solution, with a maximum increase in developed force of 10.6 ± 1.8% at low insulin concentrations (0.3 IU/L; n = 8) and 28.1 ± 5.7% (of basal twitch force, respectively) at high insulin concentrations (3 IU/L; n = 8; Figure 1B). In pyruvate-containing solution, the relative increase in twitch force with insulin was smaller but independent of the insulin concentration used (10.5 ± 3.2%, n = 8 and 13 ± 3.8%, n = 9, respectively; Figure 1B). Developed force at baseline conditions was significantly higher in the pyruvate group (50.3 ± 10.6

Figure 1. A, Original recording of effect of 3 IU/L insulin on isometric twitch tension in ventricular muscle strip from a failing human heart. After muscle was stretched to optimal length and after mechanical stabilization, insulin was added to the bathing solution (arrow). Positive inotropic effect resulted within first minutes after administration without affecting diastolic tension. B, Left, Time dependence of positive inotropic effects of 0.3 IU/L (solid bars) and 3 IU/L (open bars) insulin in glucose-containing solution; Right, time dependence of positive inotropic effects of 0.3 IU/L (solid bars) and 3 IU/L (open bars) insulin in pyruvate-containing solution. *P < 0.05 vs baseline. dev indicates developed.
versus 25.7±6.5 mN/mm² in the presence of glucose). Insulin did not significantly affect twitch kinetics (Table) but tended to prolong relaxation times.

To further assess the underlying mechanisms of the substrate dependence, additional experiments were performed after glycolysis was blocked with iodoacetate (0.5 μmol/L). In muscles preincubated with higher concentrations of iodoacetate (1 μmol/L), muscles developed contracture within the first minutes. The insulin-mediated positive inotropic effect was reduced by ~50% in the glucose group in the presence of 0.5 μmol/L iodoacetate and was comparable to the insulin-mediated effect in the pyruvate group under this experimental condition. These data show that at least some of the insulin-dependent positive inotropic effects are not related to increased glycolysis.

Additional experiments were performed in 9 trabeculae from 3 hearts obtained from patients with diabetes mellitus. Insulin exerted identical inotropic effects in both glucose- and pyruvate-containing solutions compared with nondiabetic myocardium (at 3 IU/L, 33±13% [n=6] and 15.8±11% [n=3], respectively).

**Ca²⁺-Dependent and Ca²⁺-Independent Effects of Insulin**

All further experiments designed to test the subcellular mechanisms of action of insulin were performed in glucose-containing Tyrode’s solution at an insulin concentration of 3 IU/L. First, experiments were performed in aequorin-loaded muscle strips. Figure 2A shows original tracings of the effects of insulin on twitch force and aequorin light signals. The inotropic effect of insulin (23.2±10%; P<0.05) was associated with a parallel, albeit smaller increase in aequorin light emission (15.8±4.2% of the basal value, P<0.05; Figure 2B). These data demonstrate that the functional effects of insulin are related to increased [Ca²⁺], at least in part.

We then compared these data to results from an intervention that increases force by an exclusively Ca²⁺-dependent mechanism, i.e., elevated [Ca²⁺]. Increasing [Ca²⁺], from 2.5 to 3.2 mmol/L (Figure 2B) resulted in an increase in twitch force by 23.7±4.3% (n=8; P<0.05) and an increase in aequorin light emission by 20.5±7.2% (P<0.05). Although not significantly different between groups, for an almost identical increase in force, the increase in aequorin light was smaller with insulin than with [Ca²⁺], 3.2 mmol/L. This was quantified by calculating the relative increase in force (F) versus aequorin light emission (L). ΔF/ΔL was 0.98±0.05 with elevated [Ca²⁺]₀ and 1.34±0.18 with insulin (P<0.05). These findings, in association with the tendency to prolonged relaxation kinetics, may point to an additional Ca²⁺-sensitizing effect of insulin. Interestingly, we have previously shown that IGF-1 does not affect Ca²⁺ sensitivity compared with 4 mmol/L [Ca²⁺]₀ (ΔF/ΔL was 1.02±0.38 and 1.03±0.12, respectively), but β-adrenergic stimulation reduced the relative increase in ΔF/ΔL to 0.56±0.11.

To further clarify the potential role of increased myofilament Ca²⁺ sensitivity after insulin administration, we directly assessed Ca²⁺ sensitivity in semiskinned fibers (n=5 from 4 failing human hearts). Insulin significantly shifted the tension/[Ca²⁺]₀ relationship to the left, which resulted in a decreased EC₅₀ (4.75±0.53 μmol/L) in the presence of insulin versus control (6.19±0.79 μmol/L, P<0.05; Figure 3).

### Subcellular Mechanisms of Action of Insulin

We performed rapid-cooling experiments to directly assess the effects of insulin on SR Ca²⁺ content. Figure 4A shows a representative original recording. The upper tracing reflects temperature near the surface of the muscle, the lower tracing of twitch force. At steady state isometric contractions, the muscle was cooled to ~1°C, and a stable cooling contracture as an index for SR Ca²⁺ content developed. In these experiments, we used paired cooling contractures to additionally test whether insulin directly affected SR Ca²⁺ uptake. The rationale for these experiments is that during the rewarming period (in the unstimulated muscle), cytosolic Ca²⁺ is competitively removed from the cytosol by sarcoplasmic/endoplasmic reticulum Ca²⁺-ATPase (SERCA2a) and Na⁺/Ca²⁺-exchange.
exchange. On rewarming, the muscle completely relaxed, and the experimental protocol was repeated after addition of insulin. The average data are presented in Figure 4B. Insulin (3 IU/L) significantly increased twitch force and RCCs in glucose-containing solution by 42±7.1% and 15.7±4.9%, respectively. Additional experiments were performed in 6 preparations from 6 hearts after SR function was blocked with cyclopiazonic acid and ryanodine (Figure 4B). This intervention completely prevented RCCs and reduced the insulin-dependent positive inotropic effect by ≈46%. This indicates that the inotropic effect of insulin is associated with but does not exclusively depend on increases in SR Ca2+ content.

The RCC2/RCC1 ratio in failing human trabeculae was 47% before and 47% in the presence of insulin 3 IU/L. This indicates that ≈47% of the cytosolic Ca2+ is reaccumulated into the SR, whereas ≈53% is eliminated from the cytosol via Na+/Ca2+ exchange, and that this ratio is not affected by insulin. These numbers are typical for failing human myocardium.19

To further elucidate the insulin-dependent signal transduction pathways, the functional effect of insulin was compared with the effect of 0.1 μmol/L IGF-122 without and with blockade of either the IGF-1 receptors with IGF-1 receptor antibody (α-IR-3) or PI-3-kinase with wortmannin (0.1 μmol/L). Preincubation with the antibody only blunted IGF-1-mediated inotropy, whereas wortmannin almost completely prevented the increase in twitch force after both insulin and IGF-1 (Figure 5).

Insulin-mediated positive inotropic effects were not affected by DAG-kinase inhibition (n=6) and were blocked neither by the unspecific protein kinase C (PKC) inhibitor chelerythrine (n=4) nor GF109203, which more specifically blocks typical (α,β1) and new (δ, ε) isoforms of PKC (the

Figure 3. Effect of insulin (3 IU/L) on Ca2+ sensitivity of saponin skinned trabeculae (n=5). Filled symbols indicate control; open symbols, insulin. Tension-[Ca2+] relationship is significantly shifted to the left. Mean EC50 values are 6.19±0.79 and 4.75±0.53 μmol/L for control and insulin-treated trabeculae, respectively (P<0.05). Hill slope was 2.68±0.68, respectively (P=0.99). Abscissa: 1E-7, 1E-6, 1E-5, and 1E-4 [Ca2+] (mol/l);

Figure 4. A. Original recording of rapid cooling experiment. Top depicts bath temperature; bottom, twitch force. On cooling, stable cooling contracture develops. With rewarming, the muscle completely relaxes after a brief rewarming spike. At steady state conditions, insulin (3 IU/L) is added, and the experiment is repeated. B, Left, Average values from 7 preparations from 6 hearts for experiments as shown in Figure 4A. *P<0.05 vs baseline. Right, Average values from 6 preparations from 6 hearts for experiments as shown in Figure 4A after SR function was blocked with cyclopiazonic acid (CPA) and ryanodine. *P<0.05 vs baseline, #P<0.05 vs control (ie, without cyclopiazonic acid/ryanodine).
β-adrenergic receptor stimulation in isolated failing human myocardium increases force by ~100%, and therefore, the inotropic effect observed in this study with insulin accounts for up to one fourth of the maximal response to catecholamines in the same system. In addition, the inotropic response to insulin was identical in end-stage failing myocardium from patients with long-standing diabetes mellitus.

However, we also observed a partial substrate dependency of the inotropic effect. The maximal inotropic response to insulin was ~50% smaller in pyruvate-containing Tyrode’s solution than in glucose-containing Tyrode’s solution at the higher insulin concentration. In addition, blocking glycolysis in muscles preincubated with glucose attenuated the inotropic response to insulin to the extent seen in the presence of pyruvate. We therefore suggest that the functional response to insulin may be related to 2 components: one that originates from improved glucose utilization and metabolism, and one that is independent from metabolic factors. However, we are not aware to what extent we have reduced glucose metabolism at the iodoacetate concentration used in these experiments, because higher concentrations of the inhibitor induced irreversible contracture of the muscles. Furthermore, basal developed force was significantly higher in the pyruvate group. This is in line with the described positive inotropic effect of pyruvate. With the higher basal twitch tension, an additional positive inotropic response may appear smaller if calculated as a percentage of basal force. In fact, the difference in the average increase in twitch force with 3 IU/L insulin was also less pronounced if absolute values were calculated: force increased by 4.3 mN/mm² in glucose-containing solution and by 3.3 mN/mm² in pyruvate-containing solution.

Signal Transduction Pathways of Insulin in Human Myocardium

In addition to binding to sarcolemmal insulin receptors, insulin is able to bind to both types of IGF receptors. We have previously shown that IGF-1 binding to the IGF-1 receptors in isolated guinea pig and rat hearts,3,5 even the lower concentration used in the present study. Although these concentrations are significantly lower than those used in previous studies in guinea pig and rat hearts,3,5 even the lower concentration used is up to 5 times higher than postprandial plasma insulin levels in healthy volunteers. However, local insulin concentrations at the receptor level are unknown and may be higher than plasma levels. In addition, higher plasma insulin concentrations may be reached under clinical conditions such as intravenous insulin application (eg, insulin-glucose-potassium infusions). We observed a concentration-dependent positive inotropic effect of insulin in isolated end-stage failing human myocardium that accounted to up to ~25% increase in twitch force at 3 IU/L insulin. The inotropic effect was associated with minor prolongations of relaxation time and no change in diastolic tension. The magnitude of the inotropic responses to insulin in the present study is comparable to the effects reported in isolated guinea pig and rat hearts.

The acute functional effects of insulin in the present study were substantial and may be of functional relevance: maximal improvement in ventricular performance and hemodynamics in the human peripheral and coronary circulation remains unknown. Insulin also exerts direct vasodilatory effects in the human coronary circula-

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receptors results in a Ca\(^{2+}\)-dependent positive inotropic effec-
t.\(^{22}\) Nevertheless, in the present study, insulin effects were
not significantly blunted by preincubation with the selective
IGF-1 receptor antibody a-IR-3. This demonstrates an IGF
receptor–independent signal transduction pathway for func-
tional effects of insulin in human myocardium, most likely
via direct activation of the insulin receptors. Both insulin
receptors and IGF-1 receptors initiate signal transduction
pathways that involve the insulin-receptor substrate (IRS).
Tyrosine-phosphorylated IRS consecutively activates signal-
molecules with SH2 domains, including PI-3-kinase.\(^{34,35}\)

The PI-3-kinase–dependent pathway mediates antiapoptot-
ic signaling and trophic effects via activation of Akt. The role
of PI-3-kinase activation in mediating metabolic actions of
insulin is controversial.\(^{36–38}\) In the present study, preincuba-
tion of human ventricular muscle with the selective PI-3-
kinase inhibitor wortmannin almost completely prevented the
insulin-dependent inotropic effects. Similarly, we could pre-
viously demonstrate that PI-3-kinase also mediates IGF-1–
dependent functional effects.\(^{22}\) These data suggest that PI-3-
kinase activation is a key event in the signal transduction
network activated by insulin or IGF-1 that ultimately results
in functional effects. We also tested the involvement of other
kinases, such as PKC, which may be activated downstream
of PI-3-kinase;\(^{39}\) however, neither inhibition of PKC nor inhi-
bition of DAG had any measurable effect on the inotropic
response of failing human cardiac muscle to insulin. There-
fore, the chain of events that couples insulin receptors and
PI-3-kinase activation to functional effects deserves further
investigation.

**Influence of Insulin on Intracellular Ca\(^{2+}\) Handling**
An increase in [Ca\(^{2+}\)], as the underlying mechanism for the
inotropic effect of insulin was demonstrated for rat whole-
heart preparations.\(^{3}\) Therefore, we assessed the effects of
insulin on intracellular Ca\(^{2+}\) handling in human cardiac
muscle. We could demonstrate that intracellular Ca\(^{2+}\) tran-
sients increased in parallel to twitch force in aeroin-loaded
trabeculae. Increased Ca\(^{2+}\) transients may result from in-
creased transsarcolemmal Ca\(^{2+}\) influx, increased SR Ca\(^{2+}\)
release, or both. We therefore directly tested the effects of
insulin on SR Ca\(^{2+}\) content and SR Ca\(^{2+}\) reuptake via
SERCA2a (relative to transsarcolemmal Ca\(^{2+}\) elimination)
using RCCs. We could demonstrate that the inotropic re-
sponse to insulin was associated with but not dependent on an
increase in SR Ca\(^{2+}\) content. In addition, data from the paired
rapid cooling experiments revealed that insulin did not
change the relative contribution of SERCA2a versus Na\(^+\)/
Ca\(^{2+}\) exchange for cytosolic Ca\(^{2+}\) elimination. This sup-
ports the hypothesis that insulin increases transsarcolemmal Ca\(^{2+}\)
influx without directly affecting SR Ca\(^{2+}\) handling properties.
It also supports our notion that substantial parts of the
inotropic effects of insulin are not related to metabolic
changes, which might (by improved phosphorylation poten-
tial) increase the Ca\(^{2+}\)-reuptake capacity of SERCA2a.\(^{40}\)

PI-3-kinase–dependent activation of reverse-mode Na\(^+\)/
Ca\(^{2+}\) exchange recently has been shown by our group to be
one source of Ca\(^{2+}\) entry in human myocardium.\(^{22}\) Therefore,
we directly tested the effects of pharmacological reverse-
mode Na\(^+\)/Ca\(^{2+}\) exchange inhibition on the functional effects
of insulin. In fact, this intervention partially prevented the
inotropic response of human cardiac muscle to insulin.
Therefore, a PI-3-kinase–dependent, reverse-mode Na\(^+\)/Ca\(^{2+}\)
exchange activation with consequent increased transsar-
colemmal Ca\(^{2+}\) entry during the early phase of the action
potential may be one of the mechanisms by which insulin
increases Ca\(^{2+}\) transients, SR Ca\(^{2+}\) content, and twitch force.
Nevertheless, the mechanisms that couple PI-3-kinase to
Na\(^+\)/Ca\(^{2+}\) exchange remain to be determined.

**Insulin and Myofilament Ca\(^{2+}\) Handling**
Inotropic interventions may be associated with increased,
unchanged, or decreased responsiveness of the myofilaments
for Ca\(^{2+}\).\(^{29}\) Typically, an intervention that results in an
increased myofilament Ca\(^{2+}\) responsiveness prolongs twitch
kinetics, whereas an intervention that reduces Ca\(^{2+}\) myofil-
ament sensitivity abbreviates twitch kinetics. In the present
study, the inotropic effects of insulin were associated with a
slight, albeit nonsignificant, prolongation of twitch kinetics.
Furthermore, Ca\(^{2+}\)-sensitizing inotropic interventions are
characterized by underproportional increases in intracellular
Ca\(^{2+}\) transients, whereas Ca\(^{2+}\)-desensitizing interventions
show overproportional increases in intracellular Ca\(^{2+}\) tran-
sients.\(^{29,41}\) In the present study, insulin, compared with an
increase in the extracellular Ca\(^{2+}\) concentration as a reference
intervention without effects on myofilament sensitivity,
depended on SR Ca\(^{2+}\) content relative to force increase. These 2 observa-
tions—a trend toward prolonged relaxation kinetics and
underproportional Ca\(^{2+}\)-transient increases—point to the pos-
sibility that insulin may exert additional, direct Ca\(^{2+}\)-sensitizing effects in human cardiac muscle. This hypothesis
was tested in semiskinned fiber preparations that allow the
minute experimental control of ion homeostasis on the level
of the myocytes but leave receptor-mediated signaling path-
ways operable. Indeed, in these experiments, insulin shifted
the concentration-response curve for Ca\(^{2+}\) to the left, thus
supporting the notion that the inotropic effect of insulin
depends, at least in a minor part, on enhanced Ca\(^{2+}\) respon-
siveness of the myofilaments. To the best of our knowledge,
there are no reports on direct effects of insulin on myofil-
ament Ca\(^{2+}\) sensitivity, but a wortmannin-sensitive pathway
that mediates increased myofilament sensitivity has been
described for IGF-1 by Cittadini et al\(^{42}\) in rat whole hearts.
Given the homology of the signal transduction pathways and
the wortmannin sensitivity of the insulin-mediated inotropy,
it is likely that a similar mechanism operates for both
peptides. Nevertheless, the Ca\(^{2+}\)-sensitizing effect of IGF-1
was challenged by experiments in single myocytes of failing
and nonfailing dogs,\(^{43}\) and hence, species and/or experimental
approaches (ie, loaded versus unloaded preparations) may
also play a role.

Taken together, we demonstrated for the first time a direct
inotropic effect of insulin in failing human myocardium.
Mechanistically, this functional response has 3 components,
ie, a metabolic component, a Ca\(^{2+}\)-dependent component, and
a Ca\(^{2+}\)-independent component. These interesting new as-
pects of insulin signaling in the human heart deserve further investigation.

**Study Limitations**

A limitation of the study is that aequorin light signals have not been converted to [Ca\textsuperscript{2+}]. This in itself is not a problem, but it reduces the validity of direct comparisons between different inotropic interventions.\textsuperscript{22} Although validated for human cardiac muscle,\textsuperscript{19} rapid cooling may not release all Ca\textsuperscript{2+} stored within the SR.\textsuperscript{44} In addition, RCCs are an indirect measure of SR Ca\textsuperscript{2+} content, and subcellular changes, such as the insulin-dependent increase in myofilament responsiveness to Ca\textsuperscript{2+}, may affect RCCs. KB-R7943 was used as an inhibitor of the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger reverse mode; however, KB-R7943 is not an ideal agent because it can also affect other transport systems, such as K\textsuperscript{+}, Na\textsuperscript{+}, and Ca\textsuperscript{2+} channels, and it affects Ca\textsuperscript{2+} transients even in Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger–knockout heart tubules.\textsuperscript{45} The apparent selectivity for outward versus inward Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger current is not well understood.\textsuperscript{46,47} Nevertheless, no better reverse-mode inhibitors are available presently.

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